HEAVY METAL IMMOBILIZATION OF CONTAMINATED SOIL BY WASTE AMENDMENTS

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ABSTRACT

Heavy metal-contaminated soil is one of the major environmental pollution issues all over the world. In this study four low-cost amendments, namely eggshell (inorganic), banana stem, potato peel and coconut husk (organic) were added to a slightly alkaline soil for the purpose of *in situ* immobilization of Pb, Cd, Zn and Cu, thus to modify their potential environmental impacts. The artificially metal-contaminated soil was treated with 5% inorganic amendment or 10% organic amendment for a period of 12 weeks. To simulate the rainfall conditions, a metal leaching experiment was designed and the total concentrations of the metals in the leachates were determined every two weeks by using ICP-OES. The results from the metal leaching analysis revealed that the eggshell amendment generally reduced the concentrations of Pb, Cd and Zn in the leachates, whereas the banana stem amendment was effective only on the reduction of Cd concentration in the leachates. The cumulative amount of Pb, Cd and Zn in the leachates after 6 leaching events was reduced by 14%, 83% and 70%, respectively in eggshell amended soil and the cumulative amount of Cd was reduced by 78% in banana stem amended soil. Both potato peel and coconut husk amendments did not have any positive effect on the metal leaching. A sequential extraction analysis was carried out on soils treated with eggshell and banana stem at the end of the experiment to find out the chemical speciation of the heavy metals. The findings from the sequential extraction analysis indicated that the addition of eggshell amendment notably decreased the mobility of Pb, Cd and Zn in the soil by transforming their readily available form to the less accessible fractions. The banana stem amendment also reduced exchangeable form of Cd and increased its residual form.

ABSTRAK

Tanah yang tercemar dengan logam berat merupakan salah satu isu pencemaran alam sekitar global. Dalam kajian ini, empat bahan tambahan iaitu kulit telur (bukan organik), batang pokok pisang, kulit kentang dan sabut kelapa (organik) telah dicampurkan kepada tanah yang sedikit beralkali untuk tujuan menyah-gerak unsur Pb, Cd, Zn dan Cu dalam tanah dan mengurangkan kesan logam berat ini ke atas alam sekitar. Tanah tercemar tiruan telah disediakan dengan menambahkan 5% bahan organik dan 10% bahan tak organik bagi tempoh 12 minggu. Untuk mensimulasikan keadaan hujan, eksperimen melibatkan pembebasan logam dalam larut-resapan telah direkabentuk dan jumlah kepekatan logam yang terbebas ditentukan pada setiap 2 minggu dengan menggunakan ICP-OES. Berdasarkan hasil analisis larut-resapan, tambahan kulit kentang secara amnya telah mengurangkan kandungan Pb, Cd, Zn dalam larut-resapan tersebut, tambahan batang pisang hanya efektif kepada pengurangan logam Cd dalam larut-resapan. Jumlah kumulatif kepekatan unsur Pb, Cd dan Zn selepas enam kali proses larut-resapan berkurangan masing-masing sebanyak 14%, 83% dan 70% bagi tambahan kulit telur manakala 78% pengurangan Cd berlaku bagi tambahan kulit pisang. Tambahan kulit kentang dan sabut kelapa tidak mempunyai kesan positif ke atas logam yang terbebas dalam larut-resapan. Satu pengekstrakan berperingkat telah dijalankan ke atas tanah dengan kulit telur dan batang pisang untuk menentukan penspesiesan kimia logam berat. Berdasarkan keputusan pengekstrakan, didapati bahawa penambahan kulit telur telah terbukti mengurangkan pergerakan Pb, Cd dan Zn di dalam tanah dengan menukarkan unsur-unsur ini daripada bentuk yang boleh bertukarganti kepada bentuk yang tidak boleh bertukarganti. Tambahan batang pisang turut menurunkan kesediaan Cd untuk bertukarganti dan meningkatkan Cd dalam bentuk residu.

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LIST OF SYMBOLS AND ABBREVIATIONS

– ANOVA	Analysis of Variances
– BCR	Community Bureau of Reference
– BS	Banana Stem
– CEC	Cation Exchange Capacity
– CF	Contamination Factor
– CH	Coconut Husk
– Ctrl	Control
– DOC	Dissolved Organic Carbon
– EC	Electrical Conductivity
– EG	Eggshell
– FAAS	Flame Atomic Absorption Spectrophotometry
– GCS	Geological Society of Canada
– GFAAS	Graphite Furnace Atomic Absorption Spectrophotometry
– HPDE	High Density Polyethylene
– ICP-OES	Inductively coupled plasma optical emission spectroscopy
– ICP-MS	Inductively coupled plasma mass spectroscopy
– IQ	Intelligence Quotient
– MANOVA	Multivariate Analysis of the Variance
– PP	Potato Peel
– PTFE	Polytetrafluoroethylene
– OM	Organic Matter
– SEP	Sequential Extraction Procedures
– S/S	Solidification/Stabilization

– TC Total Carbon

– TN Total Nitrogen

– U.S. EPA U.S. Environmental Protection Agency

– VEB Vertical Engineered Barriers

– WHC Water Holding Capacity

– XAFS X-ray Absorption Fine Structure

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CHAPTER 1

INTRODUCTION

1.1. Heavy metals

1.1.1. Definition

Heavy metals are considered as those metals with a density of more than 5 g cm^{-3} (Järup, 2003). The most common heavy metals in contaminated soils are Pb, Cd, Zn, Cu, Cr and Hg (EPA's Recent Developments, 1997). These heavy metals are of great concern since they pose threat to human and other living organisms due to the risks of bioaccumulation and biomagnification in the food chain (Nemati *et al.*, 2011).

1.1.2. Bioavailability

The term bioavailability of a heavy metal refers to a fraction of the total concentration of the metal in soil which is available to receptor organisms and may become involved in the metabolism of the organisms (Bioavailability of contaminants in soil, 2003). The fate and transport of a heavy metal strongly depends on its chemical forms and speciation in soil.

Metal speciation can be identified by both single extraction and sequential extraction procedures. These analytical processes basically estimate the distribution of different chemical forms of a heavy metal in soil. The sequential extraction methods have primarily been used to determine the fraction of the metals in soil and the results have been correlated with plant uptake of these heavy metals. It was confirmed that these analytical methods are successful in predicting the availability of heavy metals in soil (Adriano *et al.*, 2004).

1.1.3. Environmental remediation

Heavy metal-contaminated soil may require remediation when the concentrations of one or more metals are exceeded. Traditional methods such as excavation are expensive and environmentally destructive. Immobilization technique as a low-cost and environmental friendly method has been developed for the remediation of metal-contaminated sites (Adriano *et al.*, 2004). It has been reported that the bioavailability of heavy metals can be minimized through immobilization technique using various organic and inorganic amendments. Among various immobilizing agents, natural or waste materials and some by-products have attracted increasing attention because of their low-cost, availability and being environmentally safe (Guo *et al.*, 2006).

Eggshell is a by-product which is usually generated as a waste in a large amount in all countries. This waste material contains high level of calcium carbonate ($CaCO_3$) (Ahmad *et al.*, 2012), which may play a role as an immobilizing agent to fix the metals in the soil (Ok *et al.*, 2011a). The use of eggshell as a source of $CaCO_3$ for the immobilization of Pb and Cd in acidic soils has been reported by Ok *et al.* (2011a), Ahmad *et al.* (2012); and Lee *et al.* (2013) and the effect of the amendment was attributed to the increase in the soil pH. However, the application of this amendment as a fixing agent in alkaline soil has not yet been reported.

Banana stem and coconut husk are also commonly available and abundant natural materials. After the harvesting of banana fruits and coconut, banana stem (BS) and coconut husk (CH) are often undervalued and considered as waste materials, creating a major disposal problem (Anirudhan & Shibi, 2007). Both amendments contain cellulose, hemicellulose and tannin (Li *et al.*, 2010) which may adsorb heavy metals by forming metal complexes (Noeline *et al.*, 2005). The carboxyl groups of these compounds are the main reaction sites responsible for metal binding.

Potato peel is a starch-rich residue from the potato peeling process which contains polysaccharides, polyphenols, glycoalkaloides and etc (Schieber & Saldaña, 2009). This amendment can function as a metal binder, probably due to its content of polyphenolic compounds (Al-Weshahy & Rao, 2012). Several studies have used banana stem, potato peel and coconut husk to remediate heavy metal-contaminated wastewaters (Anirudhan & Shibi, 2007; Noeline *et al.*, 2005). However, up to date of this study, there is no report on the use of these inexpensive amendments on immobilization of metals in soil.

1.2. Background and problem description

Heavy metal-contaminated soil is one of the major environmental pollution issues all over the world, mainly arisen from anthropogenic activities. Heavy metals may enter the ecosystem through emissions from various human activities such as mine tailing, smelting and refining, disposal of hazardous wastes, agriculture, sewage sludge and improper solid waste management (Khan *et al.*, 2008; Zhang *et al.*, 2010). Excessive accumulations of metals in soil, as a result of rapid industrialization, have a high adverse biological effect on human, plant, and other living organisms (Doumett *et al.*, 2008). Moreover, unlike organic pollution, most metals do not undergo any chemical or microbial degradation. Therefore, the total concentrations of these metals persist in the environment for a long time.

Soil may act as a sink for heavy metals discharged into the environment and through this pathway the heavy metals can be transferred in the food chain (Li *et al.*, 2008). One of the major concerns on metal contaminated soil is when the metal contaminations leach to the groundwater and pollute water bodies.

To overcome the problem in dealing with heavy metal-contaminated soil, several methods have been identified such as electrokinetic, phytoextraction, and soil washing. However, in most of them, a secondary action is required as a finishing step to the

remedial process since the metal contaminations are still in the bioavailable form in the environment. For instance, in the electrokinetic technique, after the heavy metals are concentrated at the electrodes and pumped to the surface, the metal pollutants need to be disposed off properly, or in the phytoextraction technique, disposal of hazardous biomass is required after the treatment (EPA's Recent Developments, 1997). Among the widespread methods for soil remediation, *in situ* immobilization has been developed as one of the most cost-effective techniques. In this technique, a low-cost amendment is added to the soil to fix the heavy metals in the soil, thus diminishes leachability and bioavailability of the metals (Guo *et al.*, 2006; Houben *et al.*, 2012).

Heavy metals can be immobilized in soil by three mechanisms, namely adsorption, precipitation, and complexation (Farrell *et al.*, 2010). These processes of stabilization of heavy metals can be improved by adding some organic and inorganic amendments into the soil (Park *et al.*, 2011). Natural materials that are available in large quantities, and certain waste products from industrial or agricultural operations, may have a potential as an inexpensive amendments for heavy metal immobilization in soil (Gadepalle *et al.*, 2007; Guo *et al.*, 2006). Moreover, the addition of some of these amendments in the soil may improve some soil properties such as microbial and enzyme activities, or properties of soil organic matter (Janoš *et al.*, 2010).

1.3. Research objectives

In this study four low-cost amendments namely inorganic eggshell and organic banana stem, potato peel and coconut husk were used for the purpose of *in situ* immobilization of Pb, Cd, Zn and Cu in a slightly alkaline soil. The goal of the soil remediation in this study was not to remove the heavy metals from the soil, but to fix the bioavailable forms of the metals by changing the chemical properties of the soil using the abovementioned amendments. The specific aims of this study are:

- To evaluate the efficiency of selected amendments (eggshell, banana stem, potato peel and coconut husk) on the *in situ* immobilization of Pb, Cd, Zn and Cu in a contaminated soil *via* determining the changes of leachability (mobility) upon amendment application in soil;
- To compare the ability of four low-cost amendments (eggshell, banana stem, potato peel and coconut husk) on immobilization of Pb, Cd, Zn and Cu in slightly alkaline soil.
- To determine the fractionation of heavy metals (Pb, Cd, Zn and Cu) and their re-distribution after the addition of selected amendments.

The aim of the soil remediation in this study is to reduce the bioavailability of the heavy metals. To achieve the aim of this research, a metal leaching experiment was carried out to simulate rainfall conditions. Moreover a sequential extraction analysis was conducted to find out the speciation of the metals in the soil.

CHAPTER 2

LITERATURE REVIEW

2.1. Sources of heavy metal in soil environment

Heavy metals occur in soil naturally by the process of weathering and their concentrations vary based on the origin of the parent materials. However, they are observed as trace elements and rarely considered as toxic (Kabata-Pendias, 2000). Due to the anthropogenic activities, as a result of industrialization, contamination of soil has been accelerated rapidly and there is a widespread concern over this problem. The effects of different human activities such as agriculture, mine tailings and disposal of hazardous wastes on soil metal-pollution are discussed hereunder.

2.1.1. Fertilizer products

Plants need essential macronutrients such as N, P, K, Ca, and Mg, as well as, some micronutrients such as Cu and Zn to grow (Lasat, 2000). However, the high concentrations of the latter group in soil can be toxic to the plants and other living organisms.

As a matter of fact, the high agriculture productions need frequent use of pesticides, fertilizers, animal manures and biosolids which leads to an increase in the environmental metal-pollution (Adriano, 2001). To provide sufficient nutritious substances such as nitrogen and phosphorus for the agricultural soil, artificial fertilizers are usually added. These supplements contain trace amounts of some heavy metals such as Cd and Pb which do not have any physiological role within the human body. Phosphate fertilizers are well-known for containing Cd and there have been extensive attempts to reduce the accumulation of Cd in soil by way of using low Cd-contained

phosphate fertilizers (Bolan *et al.*, 2003).On top of that, these phosphate fertilizers also have other toxic elements such as Hg (Chandrajith & Dissanayake, 2009).

2.1.2. Pesticides

Several pesticides which contain different concentrations of heavy metals have been used in agriculture and horticulture. However, the use of such materials has become more localized and restricted to particular crops or sites. Examples of such pesticides are copper-containing fungicidal sprays such as `Bordeaux mixture' (copper sulfate) and copper oxychloride (Jones & Jarvis, 1981). Lead arsenate was used in fruit orchards for a long time to control various types of parasitic insects. Arsenic-containing compounds were also used to control cattle ticks and pests in banana plantations in New Zealand and Australia (McLaughlin *et al.*, 2000).

2.1.3. Biosolid and manure

The application of biosolid (sewage sludge) and manure has been widespread worldwide; however, they may cause an increase in concentration of heavy metal in agricultural soil (Silveira *et al.*, 2003).

Although the term "sewage sludge" is used in many references, the term "biosolids" is getting more common as the substitution for sewage sludge, because it is believed to reflect more precisely the beneficial characteristics inherent to sewage sludge (Silveira *et al.*, 2003). The heavy metals most commonly found in biosolids are Pb, Ni, Cd, Cr, Cu, and Zn, due to the contamination of these wastes by industrial activities (Haynes *et al.*, 2009; Silveira *et al.*, 2003). Heavy metals in manure are also derived from ingestion of metal-contaminated soil by the animals. Moreover, some metals such as As, Cu, and Zn are usually added to livestock and poultry feed to improve the feeding efficiency (Papaioannou *et al.*, 2005).

Although land application of biosolid is a common practice, the long-term use of biosolid can cause heavy metal accumulation in soils. In the United States, more than half of approximately 5.6 million tons of disposed dry sewage sludge is land applied annually. In the European community, over 30 percent of the sewage sludge is used as fertilizers in agriculture (Silveira *et al.,* 2003). In Australia, 175,000 tons of dry biosolids are produced each year by the major metropolitan water authorities (McLaughlin *et al.,* 2000). Around two thirds of all biosolids is applied to lands as a fertilizer in Australia (Darvodelsky & Bridle, 2012). There is also a considerable interest in the potential of composting biosolids with other organic materials such as sawdust, straw, or garden waste for improvement of the soil fertility (Canet *et al.*, 1998). In the USA, composting of biosolids has been increased substantially over the past 10 to 15 years; whereas in Australia and New Zealand only some biosolids composting has been tried (McLaughlin *et al.,* 2000).

2.1.4. Wastewater

The term 'wastewater' refers to the water effluent from domestic, commercial establishments and institutions, and industrial effluent which is a highly complex and nutrient-rich water (Drechsel *et al.,* 2009).

The volume of discharged wastewater has been increasing due to urbanization and industrialization. Wastewater irrigation is a widespread practice in the world (Khan *et al.*, 2008). Worldwide, it is estimated that 20 million hectares of arable land are irrigated with wastewater (Jiménez, 2006). However, wastewater contains potential toxic substances including heavy metals (Abaidoo *et al.*, 2010; Hamilton *et al.*, 2007).

Irrigating the agricultural soil with wastewater in a long-term, not only pollutes the soil, but also enhances the heavy metals uptake by plants. Hence it leads to the risk of biomagnification and bioaccumulation from food contamination. Khai *et al.* (2008)

reported that long-term wastewater-irrigation (30-50 years) of rice farm, significantly affected pH, electrical conductivity (EC), exchangeable K and Na and reverse *aqua regia*-digestible copper, lead and zinc (Khai *et al.*, 2008).

2.1.5. Metal mining and industrial wastes

Metal mining process begins with exploration and discovery of mineral deposits, followed by ore extraction, and accomplishes with closure and remediation of exploitation sites. Mining and process of metal ores are considered as significant causes of environmental pollution with heavy metals (Gosar, 2004).

Nowadays environmental impacts of mining are minimized as a result of well-designed mining operations. However, mining process can still adversely affect the environment through acid mine drainage, erosion and sedimentation, cyanide and other chemical releases, fugitive dust emissions, habitat modification, and surface and groundwater pollution (EPA's National Hardrock Mining Framework, 1997). Heavy metals that can be commonly found in the geo-environment as a result of mining process are arsenic, cadmium, copper, lead, and zinc (Nilsson & Randhem, 2008).

Industrial waste is often a significant portion of solid waste in cities. U.S. EPA reported that American industrial facilities generate and dispose approximately 7.6 billion tons of industrial solid waste each year. Other industrial activities such as petroleum, gas, coal, electrical power and nuclear power industries may also generate hazardous wastes which lead to the contamination of environment (EPA's Guide for Industrial Waste Management, 1999).

2.1.6. Air-borne sources

Generally, trace elements can be found in earth's crust and may enter the ecosystem through weathering of rocks, forest fires and volcanoes. However, the high

concentration of these elements in the environment is because of anthropogenic activities. It is estimated that mobile sources (vehicles) are the major source of airborne lead (EPA's Air and Radiation, 2012). A good example is lead emissions from motor vehicles in Australia's urban areas, which contribute about 90 percent of airborne lead (Lead alert facts, Retrieved 2013).

As a result of EPA's regulatory efforts to remove lead from on-road motor vehicle gasoline, emissions of lead from the transportation sector dramatically declined by 95 percent between 1980 and 1999, and levels of lead in the air decreased by 94 percent between 1980 and 1999. Today, the highest levels of lead in air are usually found near lead smelters. The major sources of lead emissions to the air today are ore and metals processing and piston-engine aircraft operating on leaded aviation gasoline (EPA's Air and Radiation, 2012). Some other airborne sources of heavy metals are from burning fuels, such as gasoline, oil, diesel, and wood (Ambient Air Quality Standards, 2014).

2.2. Soil chemistry and potential risks of heavy metal

There are many sources of heavy metals derived from human activities. Although, some metals are essential for human and other biological organisms, the excessive concentrations of these metals are toxic to humans, animals and plants. Bioaccumulative contaminants which are of major environmental health concerns are Hg, As, Cd, Pb, Cu, and Zn. These metals have adverse effects on human and animals health mainly through food chain (Stewart *et al.*, 2011). Hg, Pb, and Cd are the most poisonous heavy metals which are not known for any essential biological function. Therefore, they have merely harmful effects when they are up-taken from food, water, and air (Campbell, 2007). In addition, they can suppress plant growth due to their phytotoxicity. In the section below,

basic soil chemistry of Pb, Cd, Cu, and Zn followed by various potential risks of these heavy metals on human being are addressed.

2.2.1. Lead (Pb)

Pb is a bluish-white lustrous metal. It exists naturally in the environment as (i) galena (the natural mineral form of lead(II) sulfide, PbS, which is the most important lead ore mineral), (ii) deposit form of cerussite (also known as lead carbonate or white lead ore and it is a mineral consisting of lead carbonate, $PbCO_3$) and (iii) deposit form of anglesite (which is a lead sulfate mineral with the chemical formula $PbSO_4$) (EPA's Recent Developments, 1997).

Although Pb occurs naturally in the environment, the concentration of Pb has been increased because of our reliance on industrial activities. After Fe, Cu, Al, and Zn, Pb ranks fifth in industrial metal production (Manahan, 2002). Application of Pb is mainly in gasoline, batteries, pipes, pigments, solders, pewter, alloys and pesticides (Manahan, 2002; Lead alert facts, Retrieved 2013).

Both adults and children can suffer from the health effects of Pb-poisoning. However, children exposed to Pb are at higher risks especially when they are under the age of six, due to the fact that their brains are still being formed. Thus, even a very low level of Pb can lead to lowered IQ, learning deficit, short attention span, behavior disorders, stunted growth, hearing impairment, and kidney injury in young children (Lead Poisoning, 2009). At very high exposure level, lead may cause mental retardation, convulsions, coma and death (Lead Poisoning, 2009). In adults, Pb can affect various systems of the body such as nerve systems, kidneys, and reproductive systems. Accumulation of Pb in human body can increase blood pressure and also cause anemia, hence may end up in death (Lead Poisoning, 2009).

2.2.2. Cadmium (Cd)

Pure Cd is rarely found in nature, however otavite (which is a rare Cd carbonate mineral, $CdCO_3$) and greenckite (which is a rare cadmium mineral that consists of cadmium sulfide, CdS) have been discovered (EPA's Recent Developments, 1997).

This element mostly exists in the environment in a combination with other elements. As a matter of fact, Cd is a by-product from melting Zn, Pb, and Cu ores. It is usually used in metal plating and to make pigments, batteries, and plastics. It may also be present in other products including phosphate fertilizers, detergents, and refined petroleum products (Toxicological Profile for Cadmium, 2010). Cigarette smoke is another important source of Cd exposure. Cd in smokers' bodies is about twice as much as nonsmokers (Toxicological Profile for Cadmium, 2010). Long-term exposure to Cd can result in renal damage which leads to the formation of kidney stones. Acute inhalation of Cd-contaminated air may contribute to lung diseases like bronchial and pulmonary irritation (EPA's Air and Radiation, 2013).

A well-known Cd-poisoning example is itai-itai disease which was developed in people living in the Jintsu River Valley in Japan as a result of consuming rice irrigated with Cd where victims experienced proteinuria due to the chronic kidney damage as well as osteomalacia (softening of the bones) (Manahan, 2002).

2.2.3. Zinc (Zn)

Zn is a lustrous bluish-white metal which is the 23rd most abundant element in the Earth's crust. Sphalerite, also known as blende or zinc blende, is the major ore of Zn (Periodic table-Zinc, 2014). Zn occurs naturally in air, water, and soil. It is an essential element for all living organisms including human. However, the concentration of Zn in the environment has been elevated due to different anthropogenic activities (EPA's Toxicological Review, 2005). Zn is mainly used as a protective coating for iron and

steel (called galvanizing). It may also be used in alloys, batteries, pigments (plastic, cosmetic, wallpaper), construction (roofing and guttering), and rubber industry (EPA's Toxicological Review, 2005).

Zn is an essential micronutrient for human health. According to the National Pollutant Inventory (NPI) of Australia (2013), the recommended dietary allowance (RDA) for Zn is 15, 12, 10 and 5 mg a day for men, women, children and infants, respectively (Zinc and compounds, 2014). Zn shortage may result in the damaged immune system and birth defects while ingesting too much Zn causes stomachache, nausea, vomiting, skin irritation and anemia. In a longer time exposure it may also damage the pancreas, disturb the protein metabolism, and cause arteriosclerosis (Periodic table-Zinc, 2014).

2.2.4. Copper (Cu)

Cu is a metallic element that occurs naturally as free metal, or as metal complexes when associated with other elements in minerals. In the complexes, Cu exists in its +1 or +2 oxidation states. Cu is an essential micronutrient for human, animals and plants. However, according to the Australian and New Zealand Environment and Conservation Council (ANZECC) and the Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ), the recommended level for Cu is 1 mgL^{-1} of water in recreational water bodies, 65 $mgkg^{-1}$ of sediment, 1.0 - 2.5 μgL^{-1} fresh water, and 0.3 - 8 μgL^{-1} of marine water (Copper and compounds, 2014). According to the U.S. EPA, maximum contaminant level for Cu in drinking water is 1.3 mgL^{-1} (Fitzgerald, 1998).

Cu is the third most widely used metal in the world after Fe and Al (Metals_Copper, 2010). Cu is mostly used in mining and metal manufacturing, electrical equipment, batteries, construction, sewerage and water supply, pesticides,

fertilizers, industrial machinery, and vehicles compounds. Cu can enter the human body through either inhalation or ingestion. Inhaling high level of Cu may cause irritation of eyes, nose, mouth, whereas ingesting high concentration of Cu causes headache, dizziness, nausea, and diarrhea. Long-term exposure to high level of Cu, damages liver and kidneys and may lead to death (EPA'S Coppers Facts, 2008).

2.3. Reaction of heavy metal in soils

Heavy metals may interact chemically or physically with the natural compounds in the soil which may result in changing their forms of existence in the environment. These metals may be bound or sorbed by particular natural matters, which may increase or decrease the metal mobility. Reaction of heavy metals in soil is performed by different mechanisms such as adsorption, precipitation, and complexation reactions (Hashimoto *et al.*, 2009).

2.3.1. Adsorption process

Adsorption of heavy metals in soil is defined as the adhesion of these metals onto the surface of soil particles. Charged solute species (ions) are attracted to the charged soil surface by electrostatic attraction and/or through the formation of specific bonds. Retention of charged solutes by charged surfaces occurs through either specific or non-specific adsorption (Bolan *et al.*, 1999). Nonspecific adsorption is a process in which the charge on the ions balances the charge on the soil particles through electrostatic attraction, whereas specific adsorption involves chemical bond formation between the ions and the sorption sites on the soil surface (Bolan *et al.*, 2003). The most important factors which control heavy metal(loid) adsorption and their distribution between the soil and water are soil type, soil pH, metal speciation, metal concentration, solid/solution ratio and the contact time. Generally, higher metal retention and lower

metal solubility happen at high soil pH (Kent *et al.*, 2000; Martínez & Motto, 2000; Temminghoff *et al.*, 1997).

2.3.2. Complexation process

Trace metals form both inorganic and organic complexes with a range of soil components. The organic components of soil have a high affinity for metal cations because of the presence of ligands or groups that can bind to metals (Harter & Naidu, 1995). Upon increasing pH, the carboxyl, phenolic, alcoholic and carbonyl functional groups in soil organic matter deprotonate, thereby increase the affinity of ligand ions for metal cations (Bolan *et al.*, 2003). The general order of affinity for metal cations coordinated by organic matter is as follows (Adriano, 2001):

$Cu^{2+} > Cd^{2+} > Fe^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+}$

2.3.3. Precipitation process

Precipitation is considered as the predominant process of heavy metal immobilization in alkaline soil, especially where the heavy metal concentrations is significantly high. This process is mainly prevalent in the presence of sulfate, hydroxide, phosphate, and carbonate (Adriano, 2001). Hydroxide precipitation is the most common and effective method for treatment of heavy metal(loid)s (Tiinay *et al.*, 1994). Liming often increases the precipitation of metal(loid)s (Lee *et al.*, 2007). Sulfide precipitation is an efficient method for the precipitation of highly toxic heavy metal(loid)s. Metal sulfides are the least soluble minerals under reducing conditions. One attractive aspect of the sulfide precipitation is the efficacy of the method for metal(loid) removal over a wide range of pH due to low solubility of metal sulfides and fast reactions (Feng *et al.*, 2000). However, to maintain the low metal(loid) sulfides solubility, the reducing conditions need to be retained which is impractical for most surface soils (Park *et al.*, 2011). Co-precipitation also contribute in heavy metal(loid)

immobilization especially in the presence of Fe and Al (hydr)oxides (Kumpiene *et al.*, 2008).

2.4. Heavy metal-contaminated soil remediation technologies

There are several soil remediation technologies and the selection of appropriate technology is based on many factors including types and concentrations of the pollutants, soil type and structure, cost, long-term reliability, environmental disturbance, and commercial availability (EPA's Treatment Technologies, 2007). According to the U.S. EPA, soil treatment technologies are classified as (i) source control treatment and (ii) on-site containment remedies. Treatment technologies of source control can be either *in situ* or *ex situ* (EPA's Treatment Technologies, 2007). The *in situ* and *ex situ* soil remediation are defined as:

- *In situ*: treat or remove the contaminants from the soil without excavation, or removal of the soil from its original place.

- *Ex situ*: requires excavation or removal of the contaminated soil from the site or subsurface.

On-site containment remedies rely on construction of vertical engineered barriers (VEB), caps, and liners used to prevent the migration of contaminants or contaminated media.

In the following sections, four prevalent soil remediation technologies, namely electrokinetic, phytoremediation, soil washing, and immobilization are discussed.

2.4.1. Electrokinetic

Electrokinetic technique relies upon passing a low-intensity electrical current between a cathode and an anode inserted directly into the soil to mobilize contaminants in the form of ions and small charged particles. This technique is applicable in saturated soils and the addition of water might be necessary to maintain the electric current. As a result of electrolysis of the water in the soil, the pH at the electrodes turn into acidic at the anode (could be below 2) and basic at the cathode (could be above 12) (EPA's Recent Developments, 1997). The pH of the soil can also be altered by using buffer solutions. The acid fronts finally move from the anode to the cathode which results in desorption of contaminants from the soil (EPA's Recent Developments, 1997). The heavy metals can be concentrated by electroplating or precipitation at the electrodes, pumping the pollutants to the surface, or complexing with ion exchange resins (Mulligan *et al.*, 2001).

Electrokinetic technique is used in Europe to remediate soil contaminated with Cu, Zn, Pb, As, Cd, Cr, and Ni (Mulligan *et al.*, 2001). Although it has the potential to remediate soil contaminated with a wide range of pollutants, it is not a cost-effective method and can gradually change the soil characteristics, and thus the treated soil is not able to support plants growth anymore (Martin & Ruby, 2004).

2.4.2. Phytoremediation

Phytoremediation is the use of plants to remove, transfer, stabilize, or destroy contaminants in soil, sediment, or groundwater (EPA's Treatment Technologies, 2007). This is an environmentally friendly technique which has a high potential to remediate soil contaminated with more than one type of pollutant, although different species have the capability of treating the contaminated soil. Compared to some other remediation technologies, phytoremediation is a low cost technique which makes it possible to be

commercialized in the future. However, this method is suitable mostly when the concentrations of the metals are low to moderate and can only treat the soil at the root zone (EPA's Recent Developments, 1997). Wuana & Okieimen (2011) suggested the use of phytoremediation in conjunction with other more traditional remedial methods as a finishing step to the remedial process (Wuana & Okieimen, 2011).

Phytoremediation technology is classified into three different types, namely phytoextraction, phytostabilization, and phytofilteration, though the latter group is used to treat only the wastewater but not the metal-contaminated soil.

- Phytoextraction

In phytoextraction technique, hyperaccumulating plants are used to transfer the metals from the soil into different parts of the plant (roots, stems, leaves). After metal-uptake, the plant with high concentration of heavy metals is harvested, dried or composted and then either isolated as hazardous wastes or recycled as metal ore (EPA's Recent Developments, 1997). The application of crops for their fast growth rate, easy cultivation, high biomass production and their relatively high yield is commonly practiced (EPA's Recent Developments, 1997).

- Phytostabilization

Phytostabilization method uses plants to reduce the mobility and bioavailability of heavy metals in the soil. In this technique, plants which are tolerant to high level of metal concentrations are used. The function of the plant is to affect the soil pH which leads to the transformation of the pollutants to metal complexes with reduced solubility. In addition, this technique can reduce metal-leachability through controlling the soil erosion. However, phytostabilization can stabilize contaminants only in root-soil contact (EPA's Recent Developments, 1997). Therefore, plants with the deeper root systems are favored when the root systems can develop below the zone of the maximum

concentration. In contrast to phytoextraction, phytostabilization does not require disposal of hazardous biomass (EPA's Recent Developments, 1997).

- Phytofilteration

Phytofilteration also called rhizofilteration, is the use of plants to either adsorb the heavy metals onto the root surface or absorbed them by the plant roots from groundwater. The saturated roots are harvested and disposed. This technique is cost-effective for a large volume of wastewater but it is not able to treat the metal-contaminated soil (EPA's Recent Developments, 1997).

The U.S. EPA has reported the advantages and disadvantages of each types of phytoremediation technology (Table 2.1).

Table 2.1: Types of phytoremediation technology: Advantages and disadvantages (EPA's Recent Developments, 1997)

Type of Phytoremediation	Advantages	Disadvantages
Phytoextraction by trees	High biomass production	Potential for off-site migration and leaf transportation of metals to surface. Metals are concentrated in plant biomass and must be disposed of eventually.
Phytoextraction by grasses	High accumulation	Low biomass production and slow growth rate. Metals are concentrated in plant biomass and must be disposed of eventually.
Phytoextraction by crops	High biomass and increased growth rate	Potential threat to the food chain through ingestion by herbivores. Metals are concentrated in plant biomass and must be disposed of eventually.
Phytostabilization	No disposal of contaminate biomass required	Remaining liability issues, including maintenance for indefinite period of time (Containment rather than removal).
Rhizofiltration	Readily absorbs metals	Applicable for treatment of water only. Metals are concentrated in plant biomass and must be disposed of eventually.

2.4.3. Soil washing

Soil washing is one of the soil remediation method which can be done *in situ* or *ex situ*. The principle of this method is to solubilize metals in contaminated soil using water, acids, or chelating agents, hence they can be extracted. The extraction technique is accomplished by either vacuum extraction method or pump-and-treat system (EPA's Recent Developments, 1997).

Soil washing with water alone could be effective for a low concentration of contaminant; however, when the concentration of pollutants is high, other chemical agents have to be added to the water. In soil washing technique, the contaminated soil is thoroughly mixed with the aqueous solution such as surfactants, cosolvents, cyclodextrins, chelating agents, and organic acids (EPA's Engineering, 1990; Chu & Chan, 2003; Gao *et al.*, 2003). Then the cleaned soil particles are separated from the rest and the leachate is further recovered by different treatment methods such as ion exchange or activated carbon (EPA's Engineering, 1990).

The best efficiency in soil washing technique can be achieved when the soil permeability is high (Mulligan *et al.*, 2001). The cost of remediation by this method may vary depending on *in situ* or *ex situ* character, depth of contaminations, and types of washing fluid. Although, this technique can be considered as a volume reduction/waste minimization treatment process, the technique is often applied to treat organic pollution rather than heavy metals (Wuana & Okieimen, 2011; EPA's Treatment Technologies, 2007). Moreover, the contaminants are still in the environment and require further physical, chemical, and biological treatments.

2.4.4. Solidification/Stabilization (S/S)

Solidification/Stabilization technique which is referred to as immobilization or fixation, involves the addition of binding amendments to a contaminated soil to

immobilize the metals through a combination of chemical reaction, encapsulation, and reduction of permeability/surface area (Evanko & Dzombak, 1997). In this context, solidification is the physical encapsulation of pollutants in the contaminated soil by the use of some additives like cement-based and pozzolan materials while stabilization includes chemical reactions between the fixing agent and contaminants in the soil matrix (Mulligan *et al.*, 2001; EPA'S Treatment Technologies, 2007).

Vitrification is a type of S/S technique which uses thermal energy (high temperature at 1,600 to 2,000 ^{o}C) (Martin & Ruby, 2004; Mulligan *et al.*, 2001). The process involves melting the soil to form a stable, vitrified end product by the use of an electrical current which passes between electrodes. Since during the process, toxic gases like mercury are produced, vitrification has a hood to collect the volatile metals. Therefore, this technique is preferred to be applied in a soil contaminated with nonvolatile metals. Although vitrification is an expensive technique, it is the most effective method to remediate soil with mixed organic and inorganic contaminants.

The use of S/S method is common in the United States (Mulligan *et al.*, 2001). This method can be used for a wide range of heavy metal concentrations and can be applied to the depth greater than 3 meter through the use of vertical auger mixing (Martin & Ruby, 2004; Wuana & Okieimen, 2011; EPA's Recent Developments, 1997). However, some metals which do not form hydroxides, carbonates, and silicates like arsenic, chromium(VI) and mercury are not suitable for this type of remediation (Martin & Ruby, 2004; Mulligan *et al.*, 2001).

As the focus of this study is on the immobilization (stabilization) technique, the details of this technique with examples are discussed in the following sections.

2.5. Immobilization

Soil remediation by immobilization (stabilization) technique can be accomplished through both *in situ* and *ex situ* methods. *Ex situ* immobilization is applied to an excavated soil which may cause environmental contamination during the excavation, transportation and disposal. In *in situ* remediation, stabilizing amendments are added to the soil by using conventional-earth-moving equipment, vertical auger mixing, or injection grouting. It is a low cost method which requires less labor and energy compared to *ex situ* method, however, it is difficult to achieve complete and uniform mixing of agents through *in situ* immobilization (Martin & Ruby, 2004; Mulligan *et al.*, 2001; Wuana & Okieimen, 2011).

In situ immobilization technique has been used as a very promising method for remediation of heavy metal-contaminated soil (Ma *et al.*, 1993). This technique uses an exterior fixing amendment to immobilize metals in soil (Diels *et al.*, 2002). Various fixing amendments have been suggested for their ability to immobilize heavy metals in soil environment such as synthetic zeolites, phosphate-induced products, and byproducts such as biosolids and sewage sludge (Ma *et al.*, 1993).

The addition of organic and inorganic amendments to soils increases the immobilization of metal through adsorption reactions and effectively alleviates heavy metal toxicity to plants by transforming the metals into less available fractions. The incorporation of these amendments into the soil contaminated with heavy metals could maintain organic matter of soil, improve physicochemical and biological properties of soil, and increase plant production (Ok *et al.*, 2011b).

Various factors are involved in heavy metal immobilization by organic and inorganic amendments. The first and foremost parameter is pH. Most metals are less mobile in the pH of natural to alkaline (Chen *et al.*, 2000; Hodson *et al.*, 2001; Zhao & Masaihiko, 2007). Hence, in acidic soils, the addition of an alkaline amendment might

increase the soil pH, resulting in heavy metal immobilization (Fan *et al.*, 2011; Janoš *et al.*, 2010). However, in the studies conducted on alkaline soils, the effect of pH is not considered as the main parameter governing the immobilization of heavy metals in the soil (de la Fuente *et al.*, 2011; Zhou *et al.*, 2012). Other parameters including types of soil, types of amendment added to soil, metal speciation and concentration, cation exchange capacity, and organic matter content also influence the immobilization of heavy metals in soil (de la Fuente *et al.*, 2011; Domańska, 2008; Huang *et al.*, 2011; Lee *et al.*, 2011). Each of these parameters contributes in one or more immobilizing mechanisms which are discussed in Section 2.3.

Many studies have investigated the use of organic and inorganic amendments to immobilize heavy metals in soil. A list of related studies is compiled in Table 2.2 to review the use of various soil amendments with their ability to immobilize heavy metals in contaminated soils.

Table 2.2: Selected references on potential value of organic and inorganic amendments in heavy metal immobilization

Amendment	Metal(loid)s	Observations	References
$CaCO_3$, iron grit, fly ash, manure, bentonite, bone meal	Pb, Cd, Zn	All amendments lowered the concentrations of Cd and Zn in the leachates due to the increase in soil pH. Except for manure and bone meal, the other amendments reduced Pb leaching which was positively correlated with the concentration of dissolved organic carbon in the amended soils.	(Houben *et al.*, 2013)
Bone meal	Pb, Zn, Ni, Cu	The concentration of all metals decreased during the period of the study mainly due to the increase in the soil pH. The metal leaching was higher at the beginning of the experiment because of the release of highly soluble forms of the metals.	(Hodson *et al.*, 2001)
Eggshell waste	Pb, Cd	Immobilization of Pb and Cd attributed to the increase in the soil pH due to the high $CaCO_3$ content in eggshell waste.	(Ok *et al.*, 2011a)

Table 2.2 continued: Selected references on potential value of organic and inorganic amendments in heavy metal immobilization

Amendment	Metal(loid)s	Observations	References
Eggshell waste	Pb	Immobilization of Pb in amended soil due to the formation of hardly soluble $Pb(OH)_2$. The effect was ascribed to an increase in the soil pH caused by the eggshell waste.	(Ahmad *et al.*, 2012)
Cyclonic ashes	Cd, Zn	The concentrations of Cd and Zn were reduced. The authors made several hypothesis such as metal sorption and/or precipitation due to addition of the clay minerals, co-precipitation with Fe, Al, Mn.	(Ruttens *et al.*, 2010)
Blast furnace slag, alum water treatment sludge, red mud, sugar mill, green waste compost	Pb, Zn	The amendments were successful for lowering the concentrations of extractable Pb and Zn in the soil. Blast furnace slag, alum water treatment sludge and red mud induced the adsorption of Zn onto mineral surface by ligand exchange.	(Zhou *et al.*, 2012)

Table 2.2 continued: Selected references on potential value of organic and inorganic amendments in heavy metal immobilization

Amendment	Metal(loid)s	Observations	References
Coal fly ash, apatite, bentonite	Cu	The amendments reduced the water soluble/exchangeable, carbonate, Fe-Mn oxides and organically bound fraction contents of Cu but increased the residual form of Cu in soil.	(Ma *et al.*, 2012)
potassium humate, zeolite and fly ash	Pb, Zn, Cu	Mobility of Pb and Cd was decreased in potassium humate amended soil whereas mobility of Cu was reduced after addition of zeolite and fly ash. Mobility of Zn was slightly affected by the amendments.	(Janoš *et al.*, 2010)
iron grit	Cd, Zn	Metal leaching decreased by application of the amendment. The reduction reached up to 98% and 83% for Zn and Cd respectively. An increase in pH and in the number of sorption sites which bind ionic free metals and organometal complexes are possible mechanisms for this attenuation.	(Houben & Sonnet, 2010)

Table 2.2 continued: Selected references on potential value of organic and inorganic amendments in heavy metal immobilization

Amendment	Metal(loid)s	Observations	References
Cement, $Ca(OH)_2$	Pb, Cd, Zn, Co, Cu, Ni	The addition of the amendments reduced the mobility of metals at high pH, but enhanced the mobility of the metals at low pH. Metal mobilisation at high pH was observed for Cu in the acidic soil due to the liberation of dissolved organic matter.	(Hale *et al.*, 2012)
Rapeseed residue	Pb, Cd	The application of the amendment increased soil organic matter and enhanced microbial populations. The amendment also decreased the easily accessible fraction of Cd by 5-14% and Pb by 30-39% through the transformation into less accessible forms.	(Ok *et al.*, 2011b)
Rice straw ash	Cu	The addition of the amendment suppressed release of Cu into the soil which was attributed to the metal-binding. The increase in soil pH resulted transformation of Cu into less soluble forms.	(Huang *et al.*, 2011)

2.6. Determination of heavy metal concentrations in liquid and solid samples

Inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES), graphite furnace atomic absorption spectrophotometry (GFAAS), and flame atomic absorption spectrophotometry (FAAS) are used to determine the total metals concentrations in the water, aqueous or solid samples because of their utility, sensitivity, and reliability (EPA's Method 6020A, 2007). However, type of metal as well as detection limit may affect the analytical technique selection. The solid sample is basically required to be acid digested prior to analysis while the water sample is only needed to be filtered and no digestion is required. In the case of aqueous sample, it is necessary to do acid digestion before filtration (EPA's Method 6020A, 2007).

Each technique has its own strengths and limitations (Table 2.3). Generally, the ICP is widely used because it is the most powerful analytical tool for determination of trace elements. Simultaneous multi-element detection, sensitivity and throughput are the distinct characterizations of this analytical technique which make it popular among other analytical techniques.

The total concentration of metals can be determined by the use of abovementioned analytical techniques. Although total metal content present in soil gives some indication of contamination level, it may not be very informative in terms of potential environmental and human health risks. The environmental behaviour of metals in soil and potential risks to the human health depends on the forms in which metals occur in the soil. To find out the forms of metals in the soil, the sequential extraction procedure is usually required to be conducted. Therefore, this method is briefly described in the following sections.

Table 2.3: Advantages and disadvantages of ICP-MS, ICP-OES, GFAAS, and AAS

	Strengths	**Limitations**
ICP-MS	Multi-element and Excellent detection limits	Some method development skill required
	Very economical for many samples and/or elements	Higher initial capital cost
	High productivity and Wide dynamic range	Some spectral interferences, but well defined
	Isotopic measurements	Limited to <0.2% dissolved solids
	Fast semi-quantitative screening	
ICP-OES	Multi-element and Excellent detection limits	Moderate detection limits (but often much better than FAAS)
	Very economical for many samples and/or elements	Spectral interferences possible
	High productivity and Few chemical interferences	Some element limitations
	Excellent screening abilities	
	High total dissolved solids	
	Solid and organic samples	
GFAAS	Very good detection limits	Slower analysis time
	Small sample size	Chemical interferences
	Moderate price	Element limitations
	Very compact instrument	1-6 elements per determination
	Few spectral interferences Limitations	No screening ability and Limited dynamic rang
AAS	Easy to use and very fast	Moderate detection limits
	Lowest capital cost	Element limitations
	Relatively few interferences	1-10 elements per determination
	Very compact instrument and Good performance	No screening ability

2.7. Determination of heavy metals speciation in soil

Total concentration of metals in soil is informative in many areas of studies such as geochemical; however, from the agricultural or biological standpoint, the speciation (bioavailability) of these metals is more important. Tack & Verloo (1995) defined the speciation as "the identification and quantification of the different, defined species, forms or phases in which an element occurs" (Tack & Verloo, 1995). The bioavailability and mobility of a metal is governed by many factors such as soil pH, organic matter (OM) content, cation exchange capacity (CEC), total concentration of metal, and etc (Domańska, 2008; Huang *et al.*, 2011; Lee *et al.*, 2011) The more mobility of a metal leads to the higher toxicity of that to plant, animal and human.

The speciation of metal in soil can be determined *via* single reagent leaching, ion exchange resins, and sequential extraction procedures (SEP) (Zimmerman & Weindorf, 2010). In SEP, it is assumed that the most mobile metals are removed in the first fraction and continue in order of decreasing of mobility. There are many extraction procedures including Tessier Procedure, Community Bureau of Reference (BCR), Short Extraction Procedure by Maiz, Galan Procedure, and Geological Society of Canada (GSC) Procedure.

To select an appropriate SEP many factors should be taken into account such as soil type or metal-contamination level. Generally, Tessier *et al*. (1979) procedure is accepted as the most commonly used method which not only provides information about the affinity of the metals to the various soil components, but it also indicates that the chemical forms of metals, as well as, the mobility and bioavailability of metals in soil. In this method, the chemical forms of a metal is

divided into five fractions; Exchangeable, Bound to Carbonate, Bound to Iron and Manganese Oxides, Bound to Organic Matter, and Residual (Tessier *et al.*, 1979).

- Exchangeable fraction: This fraction involves those metals which are placed on the soil surface through a very weak electrostatic interaction. Therefore, they are the most soluble form and can be released readily to the environment. These metals can be replaced by neutral salts.
- Bound to carbonate fraction: Carbonate tends to be the major adsorbent for many metals when there is reduction of iron and manganese oxides and organic matter. This fraction is susceptible to changes of pH.
- Bound to Fe-Mn oxides fraction: The Fe-Mn oxides act as a coating on particles and are sensitive to the reducing condition.
- Bound to organic matter fraction: The metals can be bound to various forms of organic matters such as living organisms e.g. detritus and etc. Under oxidizing condition the organic matter can be degraded which leads to the release of those metals bound to this components.
- Residual fraction: This is the hardest fraction to be removed from the soil. The metals in this phase are likely to be incorporated into primary and secondary minerals and less likely to be released to the environment over a reasonable period of time under natural conditions.

The following chapter discusses the Methodologies involved in immobilizing heavy metals *via* the use of waste amendments.

CHAPTER 3

MATERIALS AND METHODS

3.1. Reagent

The salts $CdCl_2.H_2O$ (purity > 99%), $CuCl_2.2H_2O$ (purity > 99%), $Pb(C_2H_3O_2)_2.3H_2O$ (purity >99.5%) and $Zn(C_2H_3O_2)_2.2H_2O$ (purity > 99%) were purchased from AJAX (Australia), Merck (Darmstadt, Germany), BDH (UK) and Fluka (Switzerland), respectively. The reagents HNO_3 65%, HCl 37% and HF 40% were supplied by Fisher Chemical (U.S.). All chemicals used were of analytical grade. The standard solutions of the elements for the ICP calibration were purchased from Perkin-Elmer (U.S.). The accuracy of the soil digestion procedures was obtained using Community Bureau of Reference (BCR) standard material. Ultrapure water with a resistivity of 18.2 MΩ cm was used for all the procedures.

3.2. Soil sampling and characterization

A fresh soil sample was collected from the top soil (15 cm depth) from the Institute Science Biological farm, University of Malaya, Kuala Lumpur, Malaysia. The soil sample was air-dried for 10 days and pulverized to pass through a 2-mm plastic sieve before the following analyses were conducted.

- Particle size distribution was determined by Hydrometer Method (See Appendix A).
- pH and Electrical Conductivity (EC) were measured in a 1:5 soil to water ratio (See Appendix B).
- Cation Exchange Capacity was determined using the $BaCl_2$ Compulsive Exchange Method (See Appendix C).
- Total Organic Carbon (TC) and Total Nitrogen (TN) were determined using a CHNS analyzer (Perkin-Elmer 2400 elemental analyzer).
- Soil Organic Matter was determined by Walkley-Black Method (See Appendix D).

3.3. Soil preparation

The soil sample was artificially spiked with the following metal salts:

- $CdCl_2.H_2O$
- $CuCl_2.H_2O$
- $Pb(C_2H_3O_2)_2.3H_2O$
- $Zn(C_2H_3O_2)_2.2H_2O$

The air-dried soil was mixed with aqueous solution containing 20 mg Cd^{2+}, 300 mg Cu^{2+}, 700 mg Pb^{2+} and 900 mg Zn^{2+} per kg soil, which were prepared by dissolving their metal salts in deionised water (See Appendix E). This enrichment was based on the International Dutch Soil Standard. The applied value exceeded the Intervention Value level of heavy metals in the International Dutch Soil Standard to simulate a highly contaminated soil (Table 3.1). The treated soil was left for 3 weeks at room temperature (24-28 ^oC) to equilibrate and undergo a wetting period with soil water holding capacity (WHC) at 300 ml/kg (See Appendix F). Finally, it was air dried for 3 weeks.

Table 3.1: International Dutch Soil Standard and the applied concentrations of metals (Dutch Target and Intervention Values, 2000)

Heavy Metal	**International Dutch Soil Standard (Target Value)**	**International Dutch Soil Standard (Intervention Value)**	**Applied concentration in the experiment**
Pb (mg kg^{-1})	85	530	700
Cd (mg kg^{-1})	0.8	12	20
Zn (mg kg^{-1})	140	720	900
Cu (mg kg^{-1})	36	190	300

3.4.Amendment

In this experiment, both inorganic and organic waste amendments were sourced locally from Kuala Lumpur, Malaysia. The waste amendments namely eggshell, banana stem, potato peel and coconut husk were prepared with the following methods:

- The collected eggshells were washed thoroughly with distilled water to remove extra residues from the inner and outer surface. The cuticles were oven-dried at 105^{o}C to obtain a constant weight.
- Banana stems were repeatedly washed with distilled water to remove any dust or other impurities and sun dried for 24 hours. The biomass was then dried in an oven at 80^{o}C to reach a constant weight.
- Potato peels were washed with distilled water several times to get rid of dust and foreign particles. The potato peels were air-dried at room temperature for a period of 3 days and then oven-dried at 105^{o}C to a constant weight.
- The supplied coconut husks were dried, therefore, there was no pre-treatment for this amendment.

All the completely dried amendments were grinded by an electric dry grinder before their addition to the soil.

3.5. Leaching pot design

A leaching pot experiment was carried out to investigate the effect of eggshell, banana stem, potato peel and coconut husk amendments on the metal immobilization in the soil (Figure 3.1).

Each plastic pot (with diameter of 20 cm and a height of 12 cm) was filled with 1 kg of metal-contaminated soil. Amendments were added to the soil at 5% for eggshell

and 10% (w/w) for banana stem, potato peel and coconut husk. The percentages were selected based on the work Zhou *et al*., (2012). The treatment was performed in triplicates and a control treatment was also carried out with the same procedure but without any amendments. The added amendments were thoroughly mixed with the soil and irrigated at their WHC for a period of 30 days to equilibrate. The changes in physico-chemical properties of the soils in response to the addition of the amendments were also evaluated after stabilization period.

Two disks of filter paper (Whatman No.41) were placed at the bottom of each pot followed by a quartz wool plug to prevent coarse particles from draining out of the pot. Each pot was connected to a high density polyethylene (HDPE) container to collect the leachate from the perforated bottom. Incubation time was 90 days and a total of six leachings analysis were performed.

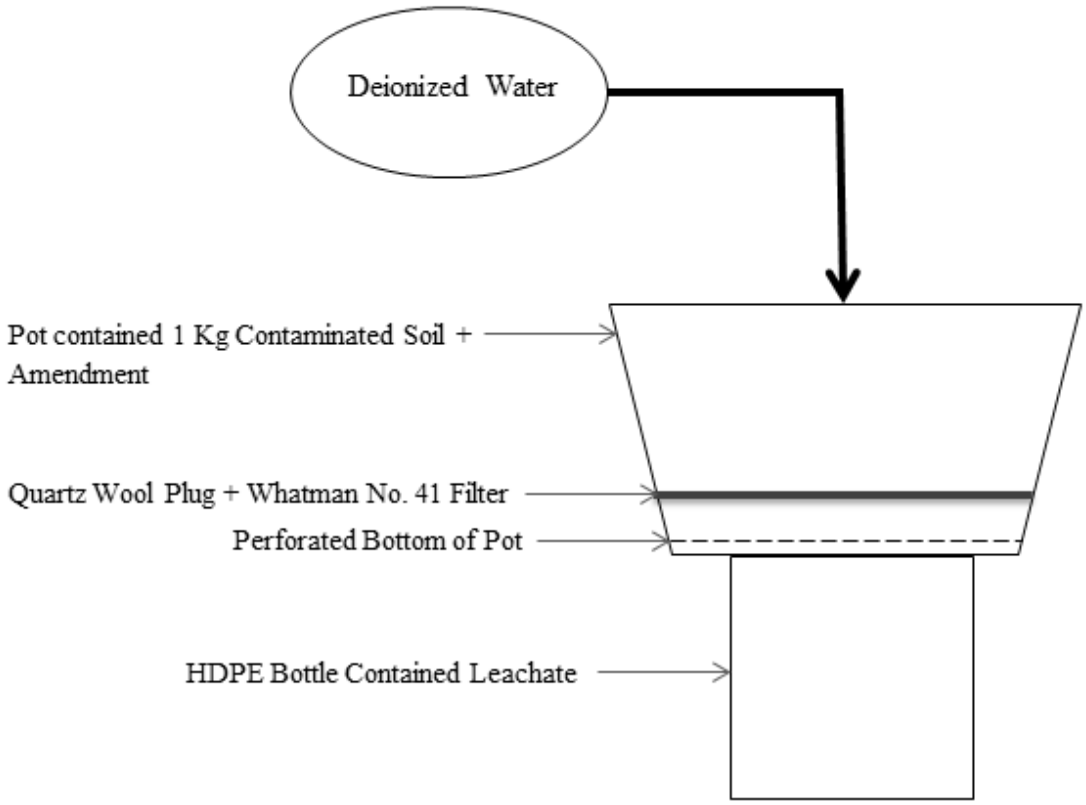

Figure 3.1: Schematic diagram of leaching pot experiment

3.6. Metal analysis

Total concentration of metals in the soil with amendment was determined by ICP-OES after acid digestion using a microwave reaction system (Anton Paar, Multiwave 3000). The soil was microwave acid-digested using the EPA's Method 3052 (HNO_3: HF: HCl, 9: 3: 2) (See Appendix G). The BCR-146R (Trace elements in sewage sludge from industrial origin) was used as a certified standard reference material. The amendments were also acid-digested according to the EPA's Method 3052 (9 mL HNO_3) (See Appendix H).

For the purpose of leachate analysis, leachates were collected every 2 weeks. A small portion of aqueous was allocated for pH determination and the rests were filtered through a 0.22 μm PTFE filter and analyzed by ICP-OES.

3.7. Sequential Extraction Procedures

The effect of each amendment on metal leachability and availability was evaluated using sequential extraction procedures according to the method proposed by Tessier *et al.*, 1979 . This method categorized metal fractionations into five types namely (i) exchangeable, (ii) bound to carbonate, (iii) bound to Fe-Mn oxides, (iv) bound to organic matter, and (v) residual.

In this extraction, 1 g of soil sample was placed in a 50-mL tube. The sample was exposed to extractants and shaken (Table 3.2). Each fraction was separated from the supernatant by centrifugation at 10 000 rpm for 30 min. The supernatant was collected, filtered and analyzed by ICP-OES. The soil were rinsed with 8 mL of deionized water and centrifuge again. The second supernatant was discarded. For the residual fraction,

the residue from step (iv) was dried and digested following the method described for the total concentrations of metals in the soil.

Table 3.2: Operating conditions for sequential extraction

	Time	Conditions	Quantity	Extractants
Exchangeable	1 hr	continuous agitation at room temp	8 mL	1 M $MgCl_2$, pH 7.0
Bound to Carbonates	5 hr	continuous agitation at room temp	8 mL	1 M NaOAc/HOAc, pH = 5.0
Bound to Fe-Mn Oxides	6 hr	96°C ± 3, occasional agitation	20 mL	0.04 M $NH_2OH.HCl$ in 25% (v/v) HOAc
Bound to Organic Matter	2hr	85°C ± 2, occasional agitation	3 mL 5 mL	0.02 M HNO_3 30% H_2O_2, pH 2.0 with HNO_3
	3 hr	85°C ± 2, intermittent agitation	3 mL	30% H_2O_2, pH 2.0 with HNO_3
	30 min	continuous agitation at room temp	5 mL	3.2 M NH_4OAc in 20% (v/v) HNO_3-diluted to 20 mL
Residual	20 min	175°C ± 5	9 mL 3 mL 2 mL	HNO_3 HF HCl

3.8. Statistical analysis

The evaluation of pH and the concentrations of Pb, Cd, Zn and Cu in leachates of the amended soils and the control treatment as a function of time were examined using Microsoft Excel. The variance and significant differences of pH in control and different treatments were analyzed by univariate analysis of variances (ANOVA). The variance and significant differences of the heavy metals concentrations in the different treatments along with time were analyzed by multivariate analysis of variance (MANOVA). Prior to MANOVA, normality and homogeneity of variances were tested and logarithmic transformation was applied to dependent variables when necessary. Pearson correlation coefficients were also computed to determine the correlations between the metal concentrations in the leachates.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Physico-chemical properties of uncontaminated soil and amendments

The physico-chemical properties of the uncontaminated soil and amendments are shown in Table 4.1. The uncontaminated soil sample had a pH of 7.83, electrical conductivity of 115 dS m^{-1} and cation exchange capacity of 2.6 cmol kg^{-1}. The soil texture was sandy with the total organic matter and total carbon and nitrogen of 1.31%, 0.4% and 0.17%, respectively. The concentration of Ca, Mn, Fe, Al, Pb, Cd, Cu and Zn in the soil samples are also presented in Table 4.1.

The low cation exchange capacity of the soil attests to its low metal immobilization ability which may result in increasing leaching of elements in areas with high rainfall. Generally, a sandy soil with little organic matter has a very low electrical conductivity and cation exchange capacity. The concentrations of the heavy metals in the soil sample were low; hence they did not affect the calculation of the amount of the metal salts added to the soil.

All four amendments had pH of slightly alkaline following the order of banana stem > potato peel > eggshell > coconut husk (Table 4.1). As expected, the concentration of Ca in eggshell amendment is much higher (42051 mg kg^{-1}) than that in the other amendments due to the presence of high amount of $CaCO_3$ in the eggshell. The Ca content of coconut husk amendment was remarkably lower (160.8 mg kg^{-1}) than that of other amendments. The organic matter content of the amendments followed the order of coconut husk > potato peel > banana stem > eggshell. The concentrations of the heavy metals in amendments were either not detectable or very low which allow the usage of these amendments in this study.

Table 4.1: Physico-chemical properties of uncontaminated soil and amendments

Parameter	Uncontaminated Soil	Eggshell	Banana Stem	Potato Peel	Coconut Husk
pH	7.83 ± 0.08	8.37 ± 0.08	8.89 ± 0.04	8.78 ± 0.07	7.54 ± 0.08
EC (dS m^{-1})	115 ± 11.5	315.3 ± 6.1	17134 ± 985	18287 ± 892	3033 ± 74
CEC (cmol kg^{-1})	2.6 ± 0.2	3.16 ± 0.2	151.62 ± 2.7	104.44 ± 0.6	26.46 ± 0.2
% OM	3.39 ± 0.2	5.16 ± 0.3	86.82 ± 0.2	90.73 ± 0.1	96.01 ± 0.5
% TC	0.4 ± 0.04	1.1 ± 0.08	6.11 ± 0.6	8.08 ± 0.4	23.69 ± 0.2
% TN	0.17 ± 0.02	0.28 ± 0.07	0.23 ± 0.03	0.58 ± 0.08	0.24 ± 0.01
Ca (mg kg^{-1})	439.8 ± 0.8	42051.6 ± 3.3	975.9 ± 0.1	948.3 ± 1.2	160.8 ± 0.3
Mn (mg kg^{-1})	118.26 ± 0.2	0.1 ± 0.001	76.7 ± 0.01	9.9 ± 0.01	8.3 ± 0.01
Fe (mg kg^{-1})	4407.4 ± 1.1	25.7 ± 0.3	ND	174.6 ± 0.2	1392.8 ± 1.2
Al (mg kg^{-1})	2846 ± 2.6	ND	ND	26 ± 0.03	ND
Pb (mg kg^{-1})	23.7 ± 0.01	ND	0.01 ± 0.01	0.01 ± 0.001	0.01 ± 0.01
Cd (mg kg^{-1})	5.2 ± 0.01	ND	ND	ND	ND
Cu (mg kg^{-1})	1.2 ± 0.04	ND	0.002 ± 0.001	0.01 ± 0.001	0.001 ± 0.001
Zn (mg kg^{-1})	4.8 ± 0.01	ND	ND	ND	0.002 ± 0.01

ND: Not detectable

4.2. Basic characterizations of amended soils after stabilization of amendments in metal-contaminated soils

As shown in Table 4.2, the addition of the metal salts to the soil sample slightly reduced the pH to 7.3, while the electrical conductivity was increased to 506 dS m^{-1}. The cation exchange capacity of the metal contaminated soil was 2.8 cmol kg^{-1}, which is similar to that of the uncontaminated soil.

Table 4.2 also shows the characteristics of the amended soils after the stabilization period. The pH values of the metal-contaminated soils after the amendment stabilization process became closer to the pH of the applied amendment. The electrical conductivity and the cation exchange capacity of eggshell treated soil were 349 dS m^{-1} and 3.1 cmol kg^{-1}, respectively which were the lowest compared to those of the other amended soils. Electrical conductivity was 2577 dS m^{-1} and cation exchange capacity was 16.8 cmol kg^{-1} in banana stem treated soil which were the highest compared to those of the potato peel and coconut husk amended soils. The high cation exchange capacity of banana stem amended soil reveals its high capability in immobilizing the heavy metals in soil. However, a single factor rarely accounts for the immobilization of heavy metals in soils. The organic matter content of coconut husk amended soil was 11% which was higher than that in other amended soils due to the high organic matter content of coconut husk amendment.

Table 4.2: Basic characterization of amended soil after 30 days stabilization

Parameter	HM-contaminated soil (Control)	EG amended soil	BS amended soil	PP amended soil	CH amended soil
pH	7.35 ± 0.08	8.29 ± 0.04	8.68 ± 0.05	8.51 ± 0.11	7.51 ± 0.10
EC (dS m^{-1})	506.0 ± 25	349.0 ± 7.8	2577.0 ± 26	1764.7 ± 31	648.7 ± 62
CEC (cmol kg^{-1})	2.8 ± 0.03	3.1 ± 0.33	16.8 ± 3.25	13.8 ± 0.68	3.9 ± 0.66
%OM	3.45 ± 0.23	4.05 ± 0.37	9.20 ± 0.46	7.47 ± 0.30	10.98 ± 1.05
%TC	0.42 ± 0.07	0.88 ± 0.08	1.13 ± 0.11	1.22 ± 0.22	5.77 ± 0.67
%TN	0.14 ± 0.03	0.18 ± 0.02	0.21 ± 0.03	0.23 ± 0.02	0.2 ± 0.03

HM: heavy metal, EG: eggshell, BS: banana stem, PP: potato peel, CH: coconut husk, Ctrl: Control

4.3. Effect of amendments on pH of leachates

The leachates of the amended soils were analyzed during the three-month incubation period. The value of pH and the concentration of metal in the amended soils were compared to those of the control treatments (unamended soil). The effect of four amendments, eggshell, banana stem, potato peel and coconut husk, along with the control treatment on the pH of the leachates are presented in Figure 4.1.

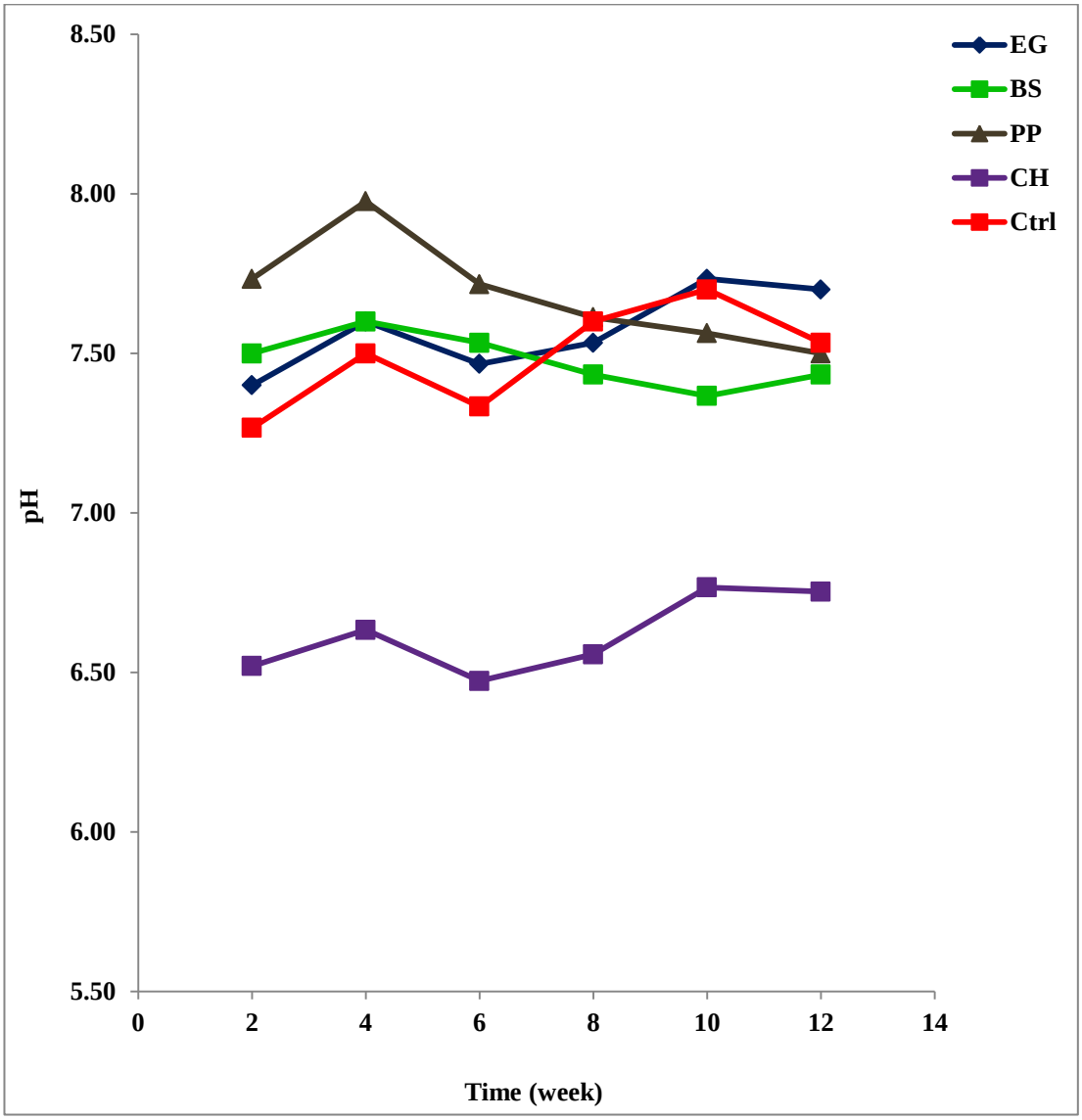

Figure 4.1: Evaluation of pH in the leachates of the eggshell (EG), banana stem (BS), potato peel (PP) and coconut husk (CH) amended soils and the control treatment as a function of time. Each point represents the average of three replicates.

The pH of the leachates was slightly alkaline, ranging from pH 7.2 to pH 7.7 in both eggshell treated soil and control treatment samples. The results revealed that eggshell amendment did not have any statistically significant effect on the pH of the leachate during the 12-weeks of the experiment.

The application of banana stem amendment significantly ($P < 0.05$) increased the pH of the leachates at Week 2 by 4% and Week 6 by 3%; whereas it significantly ($P < 0.05$) decreased the pH at Week 10 by 5% compared to that of the control samples.

The addition of the potato peel amendment significantly ($P < 0.05$) increased the pH of the leachates from Week 2, Week 4 to Week 6 by 7%, 5% and 6%, respectively compared to that of the control. From Week 6, pH of the potato peel treated soil leachate had a reducing trend and it was lower than that of the control treatment at Week 10 by 3%.

In contrast to other amendments, the addition of the coconut husk significantly ($P < 0.05$) decreased the pH of the leachates compared to that of the control samples. The leachate of the coconut husk treated soil has become slightly acidic. The amendment specifically reduced the pH by 10% at Week 2, 12% at Week 4, 12% at Week 6, 14% at Week 8, 12% at Week 10 and 10% at Week 12.

According to ANOVA Test (Post-Hoc), the addition of 5% eggshell or 10% banana stem amendments did not have any significant effect on the pH of the leachates during the period of the experiment. Thus, the leachates of both soil-amended treatments and the control treatment were slightly alkaline, ranging from pH 7.2 to 7.7, during the whole experiment. On contrary, the addition of 10% potato peel or coconut husk amendment had significant effects on the pH of the leachates.

pH is one of the key parameters which determines the metal immobilization in the soil and metals are less bioavailable in the soil with the pH of neutral to alkaline (Chen

et al., 2000; Hodson *et al.*, 2001; Zhao & Masaihiko, 2007). However, it should be noted that in acidic soils, the increase in the soil pH caused by the addition of the amendments may be considered as the main factor controlling the immobilization process in the soil (Fan *et al.*, 2011; Janoš *et al.*, 2010) but in alkaline soils, the addition of the amendments may not raise the soil pH, yet immobilize the heavy metal through other immobilizing mechanisms (de la Fuente *et al.*, 2011; Zhou *et al.*, 2012). Therefore, the alkaline nature of the soil sample in this study suggests that the effects of addition of eggshell, banana stem and potato peel amendments on heavy metal leaching is not due to changes in pH but other immobilizing factors such as type of the soil, type of the amendment, concentrations of heavy metal and metal speciation are involved.

The addition of coconut husk amendment decreased the pH of leachates compared to that of the control samples; hence, it is expected to observe an increase in the metal leaching. The decrease in the soil pH is probably due to the decomposition of the organic components of coconut husk in the soil, of which released organic acid such as humic acid.

4.4. Effect of amendments on metal concentrations in leachates

The effectiveness of four amendments, eggshell, banana stem, potato peel and coconut husk, on immobilization of Pb, Cd, Zn and Cu in the soil during the 12-weeks of the experiment are discussed in the following sections:

4.4.1. Effect of eggshell amendment on metal concentrations in leachates

The results of the leachates analysis revealed that the addition of eggshell amendment was effective in reducing Pb, Cd and Zn leaching but not on Cu leaching. The details of the obtained results are discussed hereunder.

The concentration of Pb in the leachates of the eggshell treated soil and in that of the control treatment are shown in Figure 4.2. The results revealed that the addition of 5% eggshell decreased the concentrations of Pb in the leachates by 10% at Week 2. Although Pb leaching was fluctuated during the period of the experiment, it was decreased by 28% at the end of the experiment compared to that of the control.

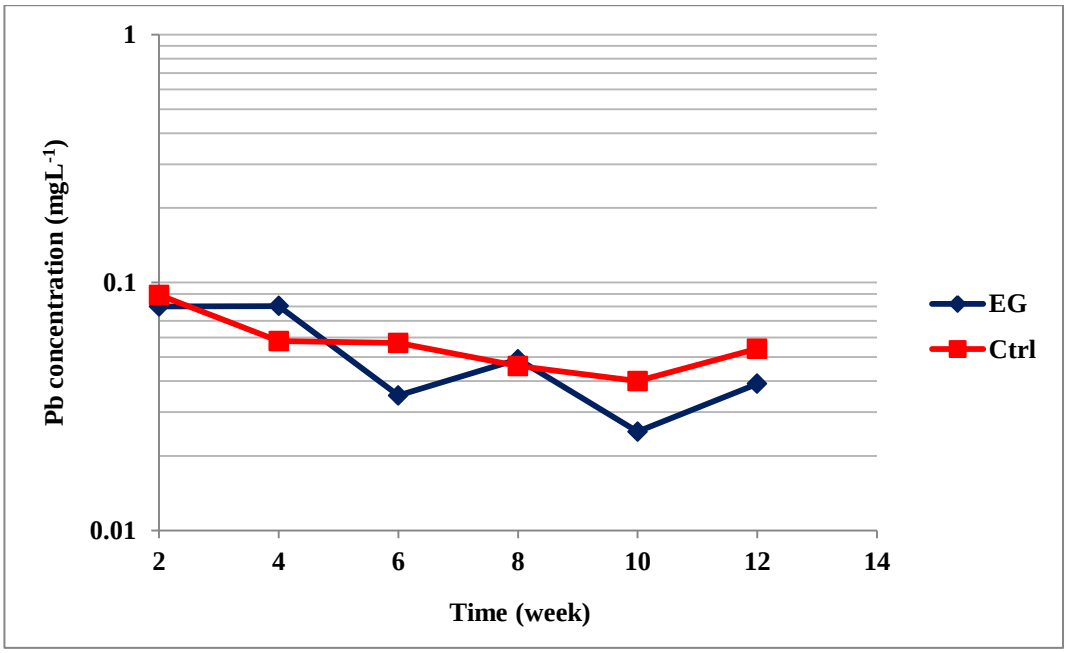

Figure 4.2: Effect of eggshell on concentration of Pb in leachates as a function of time. Each point represents the average of three replicates.

Figure 4.3 shows the effect of eggshell amendment on Cd leaching during the 12-weeks experiment. The treatment with eggshell at 5% significantly ($P < 0.05$) reduced the concentrations of Cd in the leachates during the whole experiment compared to those of the control treatment. Specifically, it reduced Cd concentration by 82% at Week 2, 85% at Week 4, 84% at Week 6, 83% at Week 8, 88% at Week 10 and 81% at Week 12, compared to those of the control treatment.

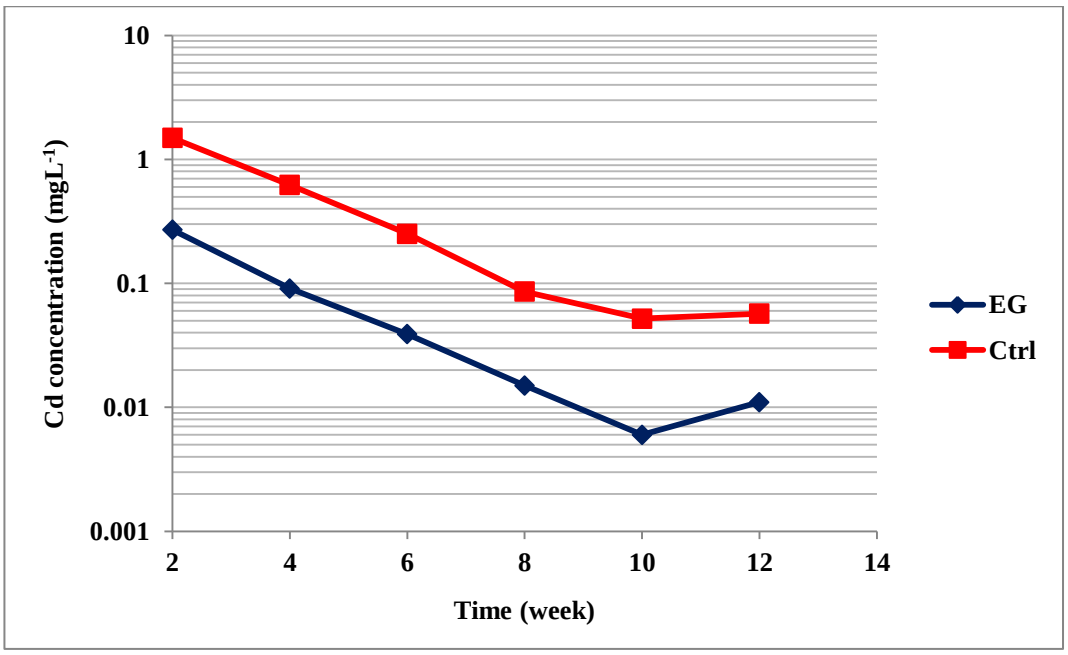

Figure 4.3: Effect of eggshell on concentration of Cd in leachates as a function of time. Each point represents the average of three replicates.

The concentration of Zn in the leachates of eggshell amended soil and the control treatment during the 12-weeks experiment are presented in Figure 4.4. The eggshell amendment significantly ($P < 0.05$) decreased the concentration of Zn in the leachates by 70%, 72%, 69%, 64%, 81% and 65% at Week 2, until Week 12, respectively, compared to those of the control treatment.

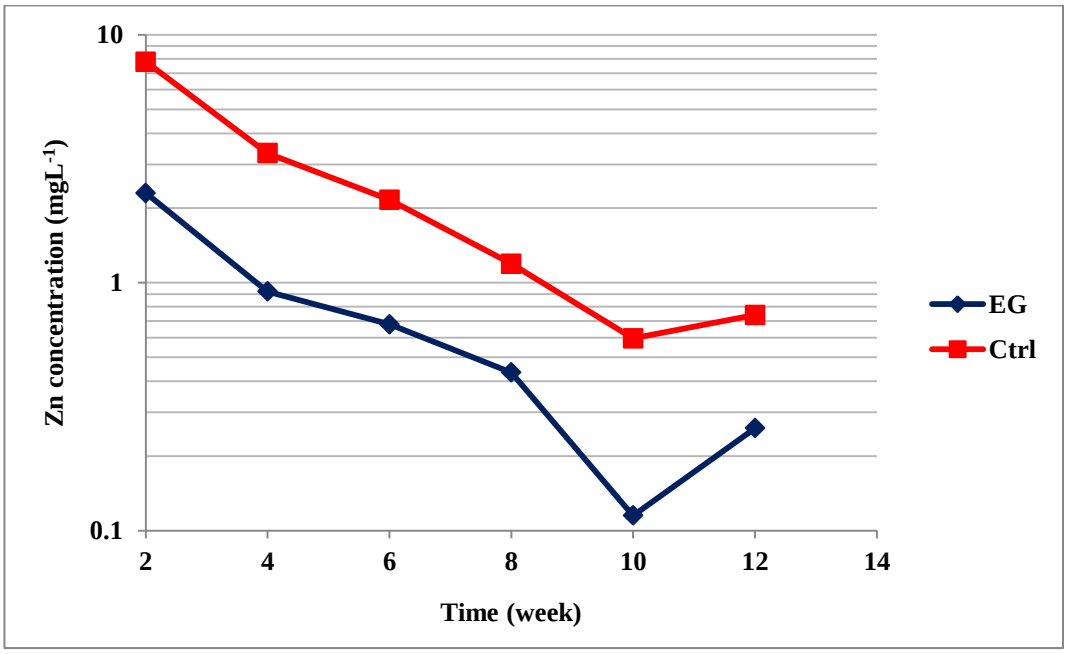

Figure 4.4: Effect of eggshell on concentration of Zn in leachates as a function of time. Each point represents the average of three replicates.

Figure 4.5 indicates the effect of eggshell amendment on Cu leaching. The addition of eggshell did not have any positive effect on the reduction of Cu leaching. The concentration of Cu was significantly ($P < 0.05$) increased in the leachates of eggshell amended soil at Week 2 by 63% and Week 6 by 45%, compared to those of the control.

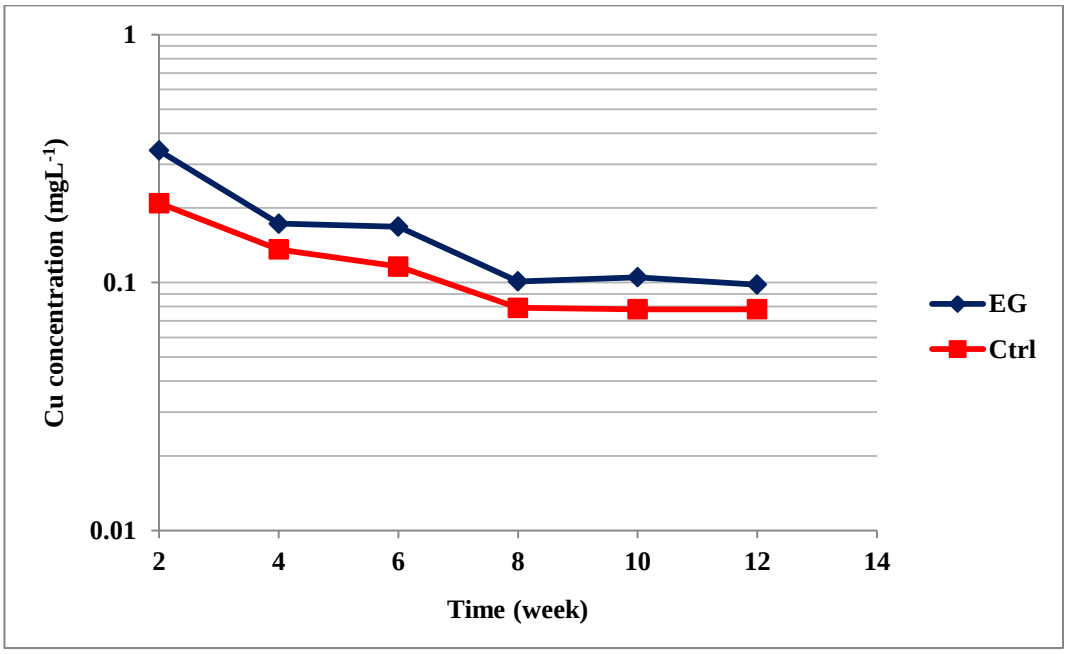

Figure 4.5: Effect of eggshell on concentration of Cu in leachates as a function of time. Each point represents the average of three replicates.

Co-precipitation may contribute to heavy metal immobilization, especially in the presence of Fe and Al hydroxides. The low concentrations of Fe and Al in the leachates of the eggshell amended soil compared to the control (Figure 4.6) indicate that these metals can be retained in the amended soil and did not wash out into the leachates. Hence, the strong decrease in Pb, Cd and Zn concentrations in the leachates is likely due to the co-precipitation of the heavy metals with Fe and/or Al (hydr)oxide which is an important mechanism in the immobilizations of the heavy metals in the eggshell amended soil (Kumpiene *et al.*, 2008).

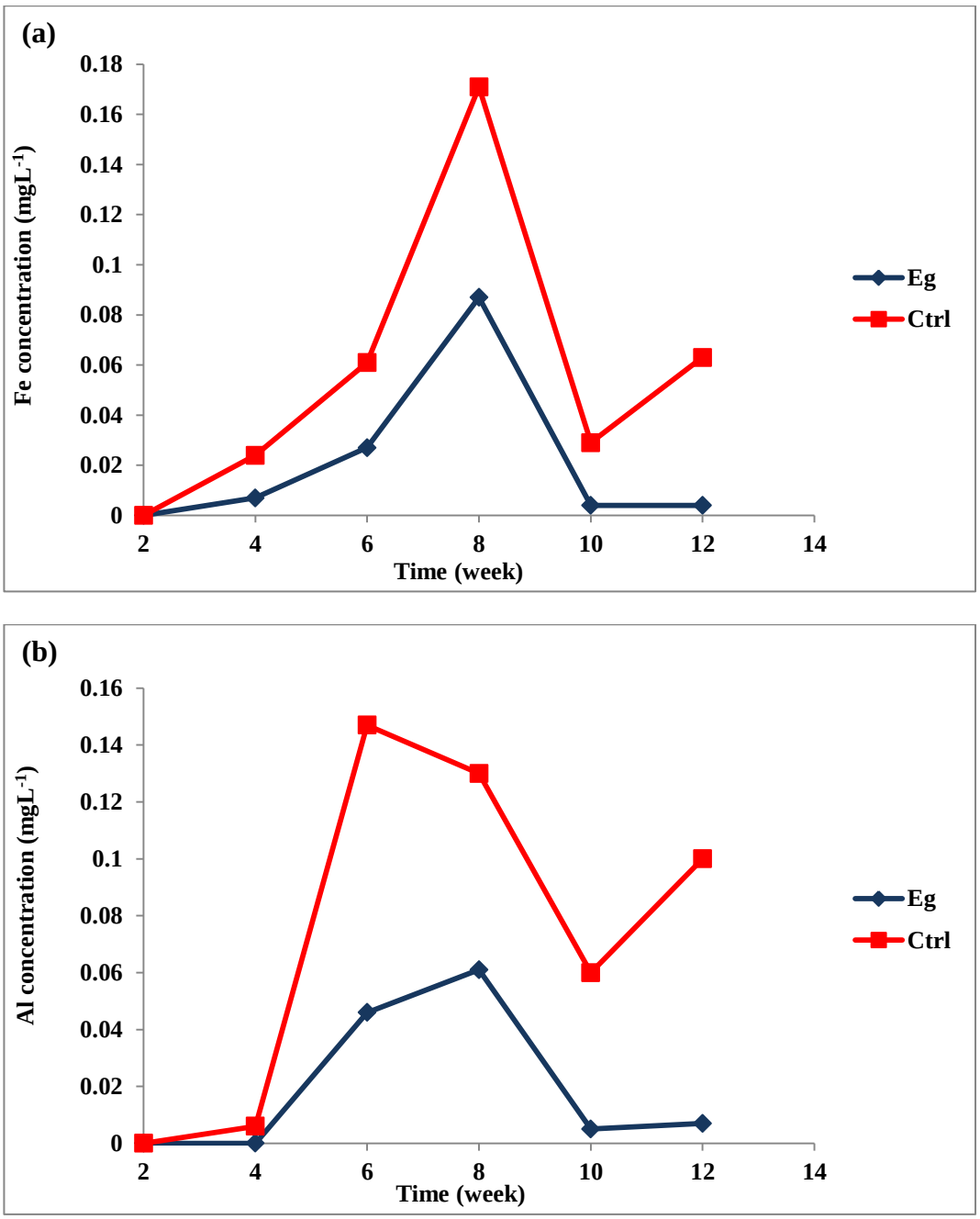

Figure 4.6: Effect of eggshell on concentration of (a) Fe and (b) Al in leachates as a function of time. Each point represents the average of three replicates.

In the related studies, immobilization of heavy metals by eggshell amendment in acidic soils was explained based on the increase in the soil pH caused by the amendment (Ahmad *et al.*, 2012; Ok *et al.*, 2010). However, in this experiment, the soil was alkaline itself and the addition of eggshell amendment did not alter the pH of the leachates. Therefore, it can be concluded that other parameters such as type of the soil, contact time, concentrations of the metals in the soil and metal speciation were

governing the immobilization of the heavy metals by the eggshell amendment. Other studies also suggest that such mechanisms can affect metal immobilization in soil (Houben *et al.*, 2013; Ma *et al.*, 2012; Ruttens *et al.*, 2010).

4.4.2. Effect of banana stem amendment on metal concentrations in leachates

The effect of the banana stem amendment on Pb leaching can be inferred from Figure 4.7. The results revealed that the treatment with the banana stem at 10% significantly ($P < 0.05$) increased the concentrations of Pb at Week 2 by 8 times and Week 4 by 40 times, compared to those of the control. From Week 4 onward, Pb concentration in the leachates of the amended soil had a decreasing trend; however, it was still remarkably higher than that of the control.

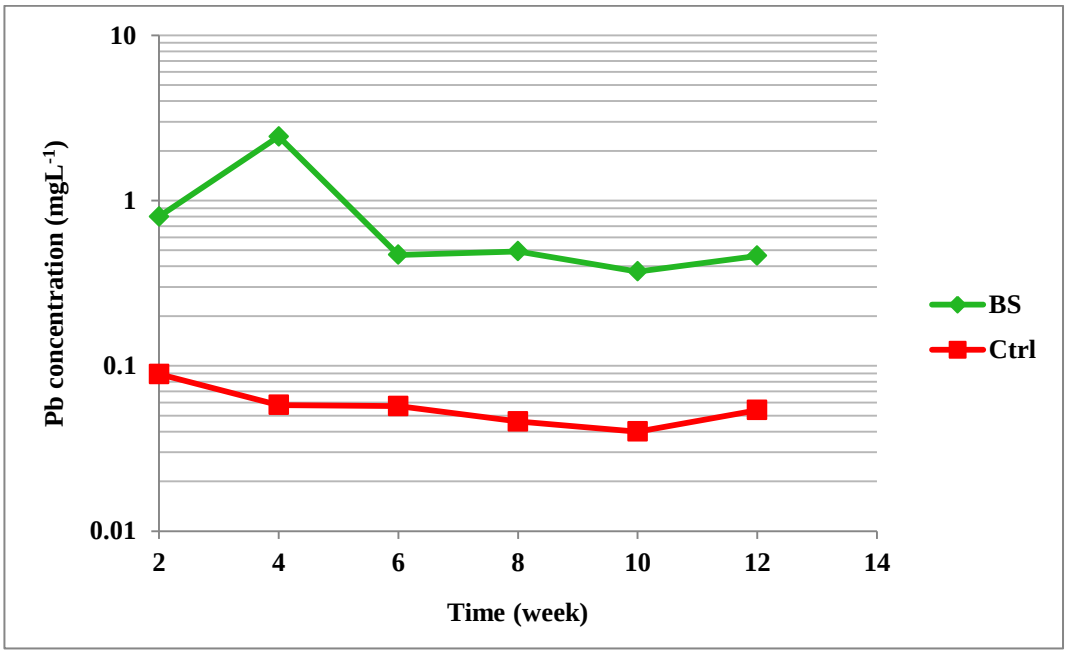

Figure 4.7: Effect of banana stem on concentration of Pb in leachates as a function of time. Each point represents the average of three replicates.

Figure 4.8 shows the effect of 10% banana stem on Cd leaching. The addition of banana stem significantly ($P < 0.05$) decreased the concentrations of Cd in the leachates at Week 2 and Week 4 by 93% and 57%, respectively compared to those of the control.

The reduction of Cd concentrations by the banana stem amendment was continued until Week 12 and it was still higher than that of the control during the whole experiment. The lowest Cd leaching in the amended soil was observed at Week 12 which was 26% lower than that of the control.

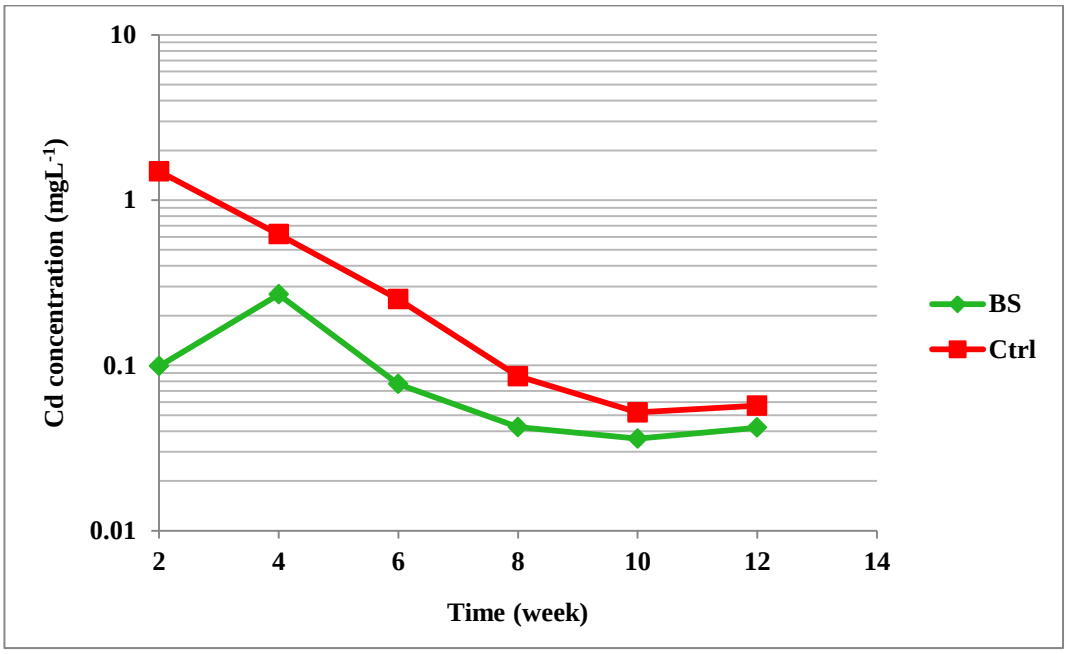

Figure 4.8: Effect of banana stem on concentration of Cd in leachates as a function of time. Each point represents the average of three replicates.

The concentration of Zn in the banana stem treated soil and the control treatment are presented in Figure 4.9. The results show that the addition of banana stem had a fluctuate effect on Zn leaching. The amendment had a significant ($P < 0.05$) positive effect on reducing Zn leaching at the first sampling by 76% but its effect was negative at Week 4 (34%). Although Zn leaching in the amended soil in comparison with the control, decreased by 46% at Week 6 and 18% at Week 8, it was slightly higher than that of the control at the end of the experiment (39%).

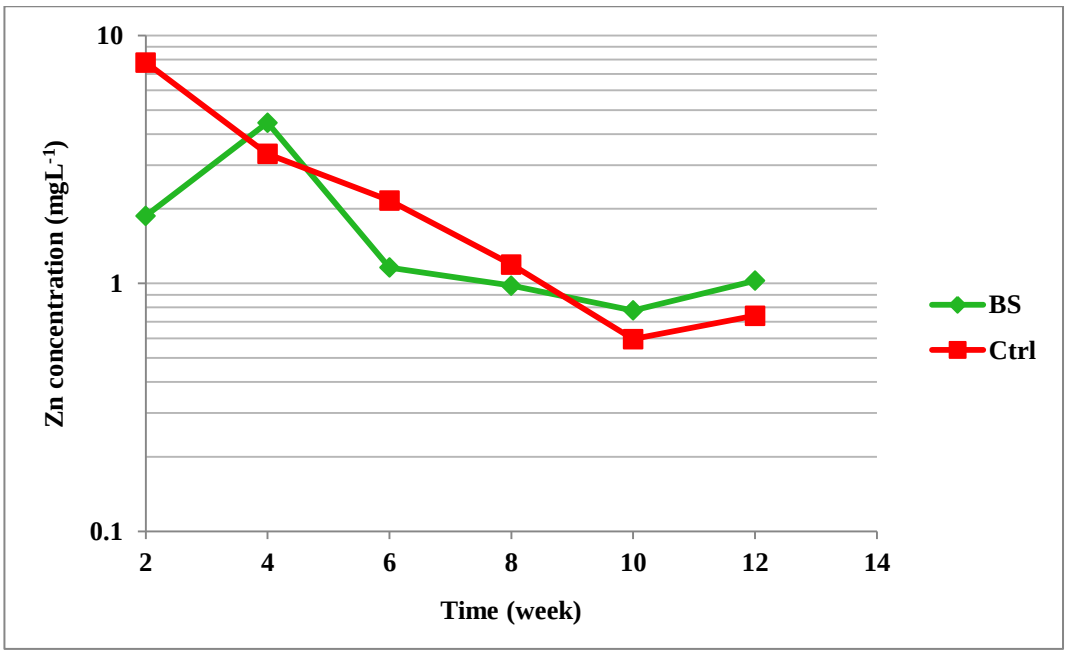

Figure 4.9: Effect of banana stem on concentration of Zn in leachates as a function of time. Each point represents the average of three replicates.

The effect of banana stem amendment on Cu leaching is shown in Figure 4.10. The addition of banana stem increased Cu leaching within 12 weeks experiment compared to those of the control. The effect was statistically significant ($P < 0.05$) at Week 2 by 7 times, Week 4 by 27 times and Week 6 by 8 times, compared to the control treatment.

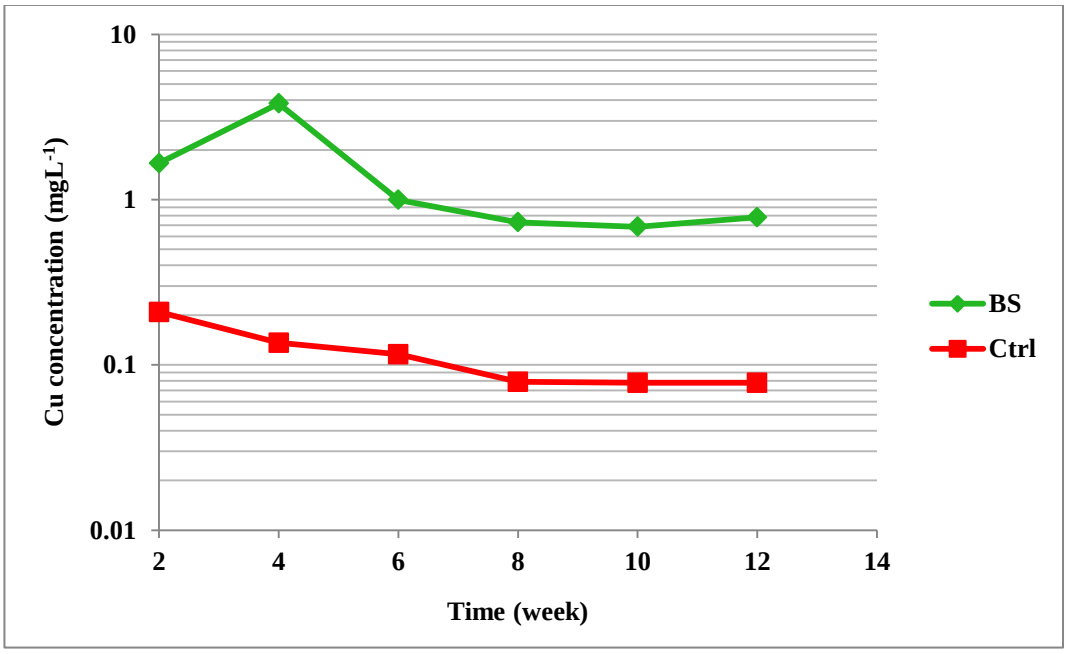

Figure 4.10: Effect of banana stem on concentration of Cu in leachates as a function of time. Each point represents the average of three replicates.

The addition of banana stem amendment into the soil increased Fe and Al leaching compared to those of the control treatment (Figure 4.11). Similarly, the addition of this amendment induced a considerable leaching of Pb, Zn and Cu from the treated soil so that, the more Fe and Al released from the soil, the more Pb, Zn and Cu released to the leachates. Therefore, the co-precipitation in the soil did not occur, but probably the heavy metals are associated with Fe and Al in soluble forms. The immobilization effect of banana stem amendment on Cd occurred due to the formation of highly insoluble Cd-complex or adsorption of Cd onto the banana stem.

As it was mentioned in Section 4.3, other parameters rather than only pH can affect the heavy metal immobilization in soil. This may also explain why eggshell and banana stem amended soils of similar pH had different effects on the metal immobilization. Similar observation was also reported by Houben *et al.* (2012) who stated that other parameters rather than only pH influence the metals immobilization in the amended soils.

One of the possible reasons for this observation might be the lower concentration of Ca in banana stem amended soils compared to that of eggshell amended soil. Ca in its compounds like CaO may be replaced with heavy metal in soil, leading to the heavy metal immobilization (Ahmad *et al.*, 2012). Another reason for the observed different effect on metal leaching might be the high leaching of Fe in banana stem amended soil compared to that of eggshell amended soil resulting in high Pb and Zn leaching.

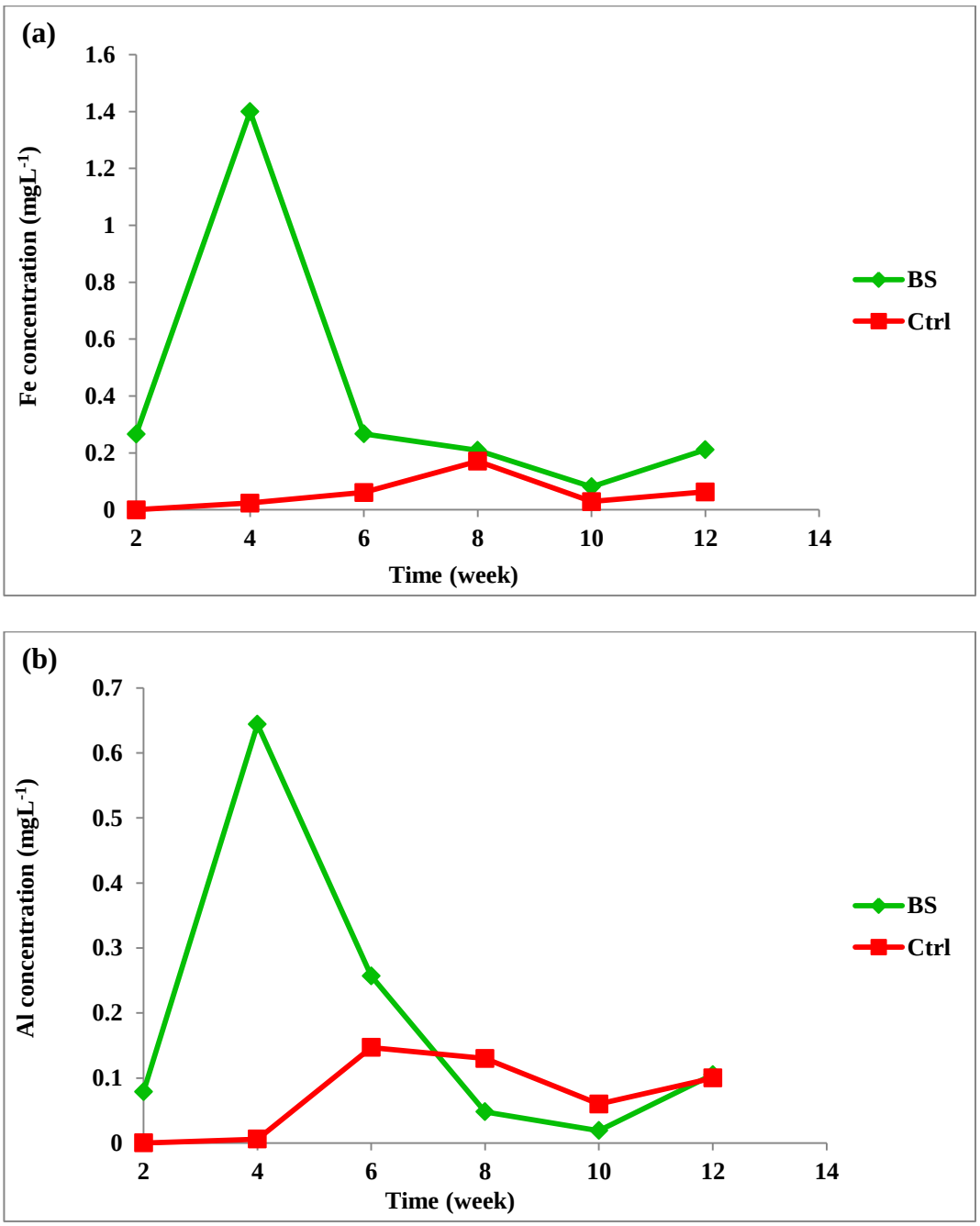

Figure 4.11: Effect of banana stem on concentration of (a) Fe and (b) Al in leachates as a function of time. Each point represents the average of three replicates.

4.4.3. Effect of potato peel amendments on metal concentrations in leachates

Figure 4.12 indicates the effect of potato peel amendment on Pb leaching. The addition of 10% potato peel significantly ($P < 0.05$) increased Pb leaching from Week 2 by 21 times to Week 8 by 20 times, compared to those of the control. After Week 8, Pb leaching in potato peel amended soil was still higher than that of the control by 16 times and 13 times at Week 10 and Week 12, respectively.

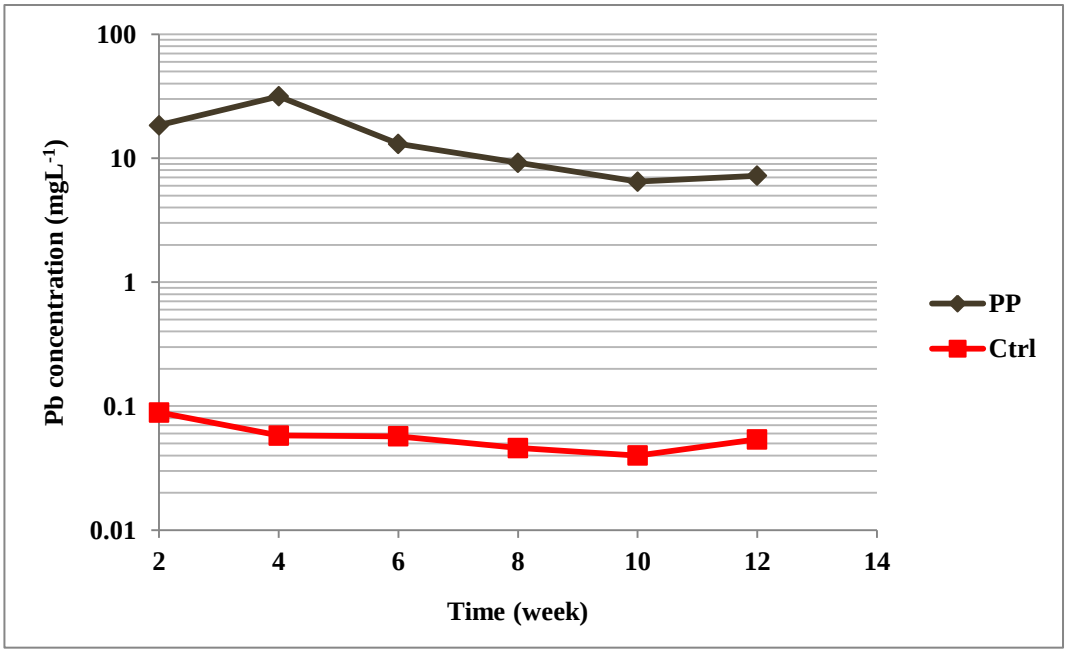

Figure 4.12: Effect of potato peel on concentration of Pb in leachates as a function of time. Each point represents the average of three replicates.

The effect of the potato peel amendment on Cd leaching is presented in Figure 4.13. The potato peel amendment significantly ($P < 0.05$) decreased the concentrations of the Cd in the leachates at the first sampling by 53% compared to that of the control. However, the amendment had a significant ($P < 0.05$) negative effect on reducing Cd leaching at Week 4 (148%). For the rest of the experiment, Cd leaching in the potato peel treated soil was higher than that of the control.

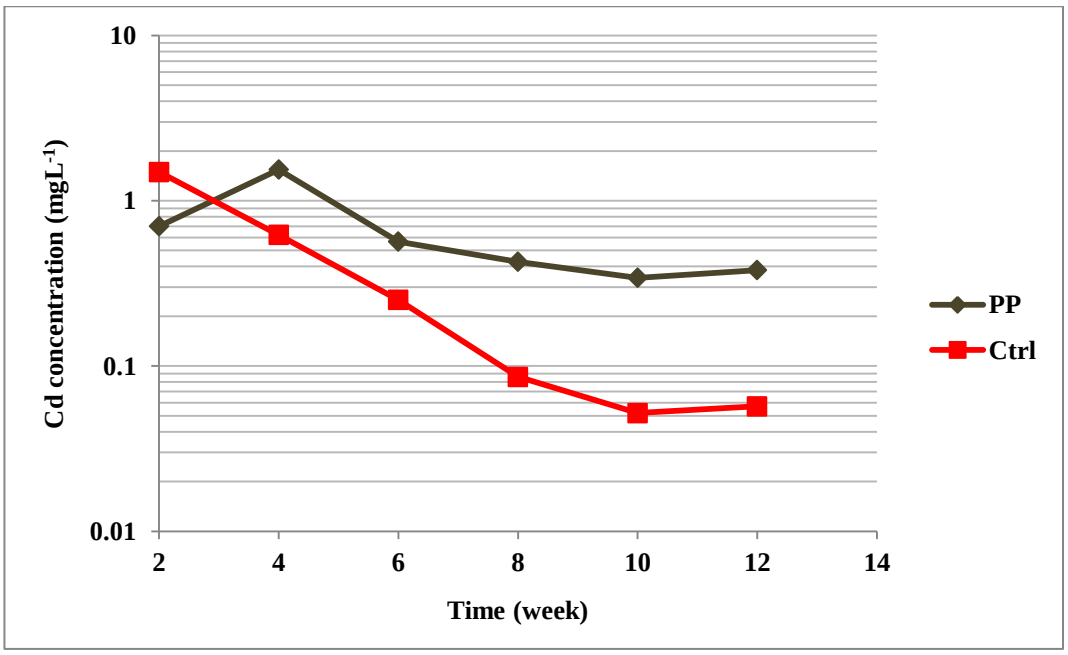

Figure 4.13: Effect of potato peel on concentration of Cd in leachates as a function of time. Each point represents the average of three replicates.

The effect of the potato peel amendment on the concentrations of Zn in the leachates in 12-week experiment is presented in Figure 4.14. Zn leaching was significantly ($P < 0.05$) increased by the potato peel amendment at Week 2 by 200%, Week 4 by 1301% and Week 6 by 611% as compared to those of the control.

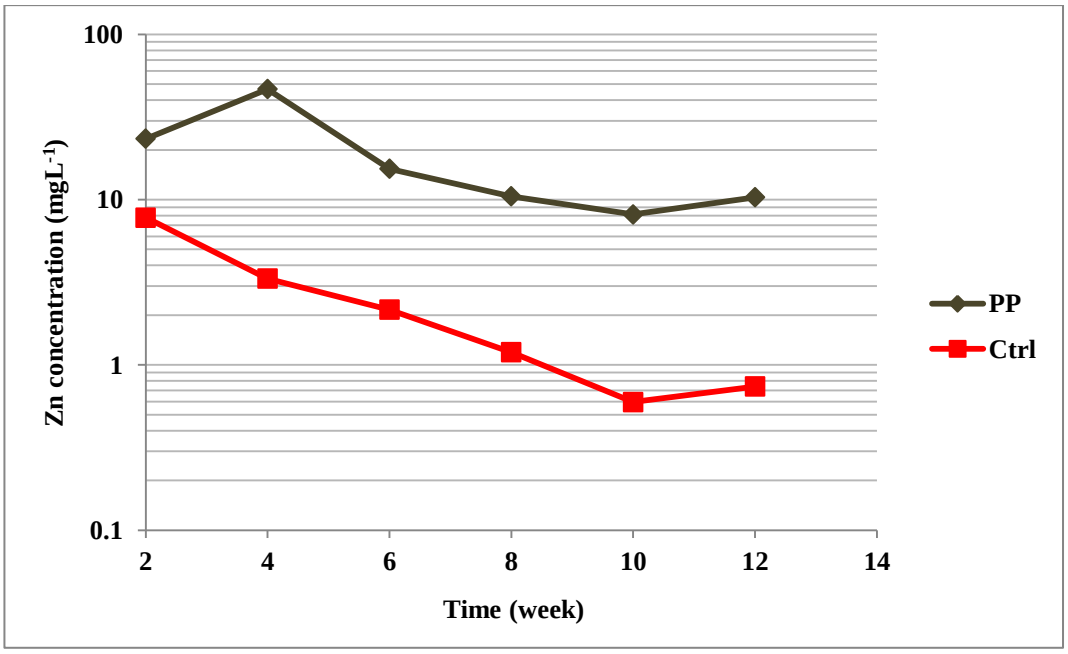

Figure 4.14: Effect of potato peel on concentration of Zn in leachates as a function of time. Each point represents the average of three replicates.

Figure 4.15 shows the effect of potato peel on Cu leaching. The results indicate that potato peel amendment had a negative effect on reducing Cu leaching. It has significantly ($P < 0.05$) increased Cu leaching within the whole experiment (except Week 12) by 22 times at Week 2, 59 times at Week4, 32 times at Week 6, 29 times at Week 8 and 24 times at Week 10, compared to those of the control treatment. At Week 12, Cu concentration in potato peel amended soil was still 20 times higher than that of the control.

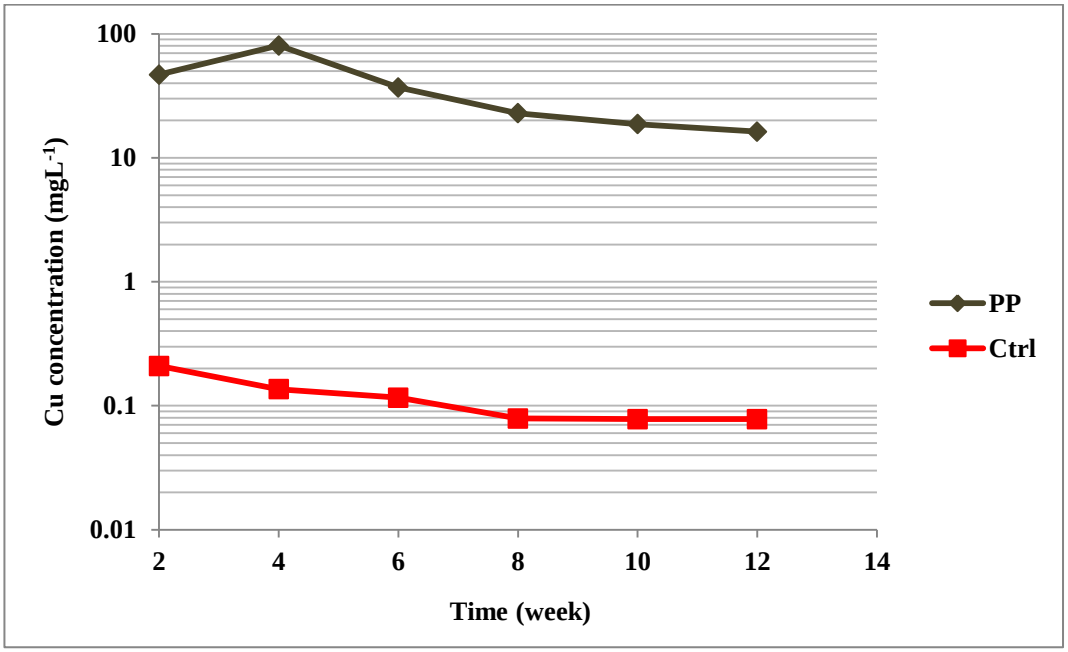

Figure 4.15: Effect of potato peel on concentration of Cu in leachates as a function of time. Each point represents the average of three replicates.

The leaching behavior of all the metals was similar in the potato peel amended soil. The potato peel amendment did not have any positive effect on the metal leaching reduction but it increased the leaching of the metals.

Since the pH of potato peel amended soil leachate was higher than the pH in the control treatment leachate, a reduction in metals leaching was expected. However, as discussed in Section 4.3, the increase of pH in alkaline soils may not determine the metal immobilization. Similar to banana stem amendment, potato peel amendment induced an increase in Fe and Al leaching (Figure 4.16) However, Fe and Al leaching in potato peel amended soil was significantly higher than those in banana stem amended

soil. This explains why although this amendment induced a similar pH to that of banana stem amendment, it was not as effective as banana stem in immobilizing the heavy metals in the soil.

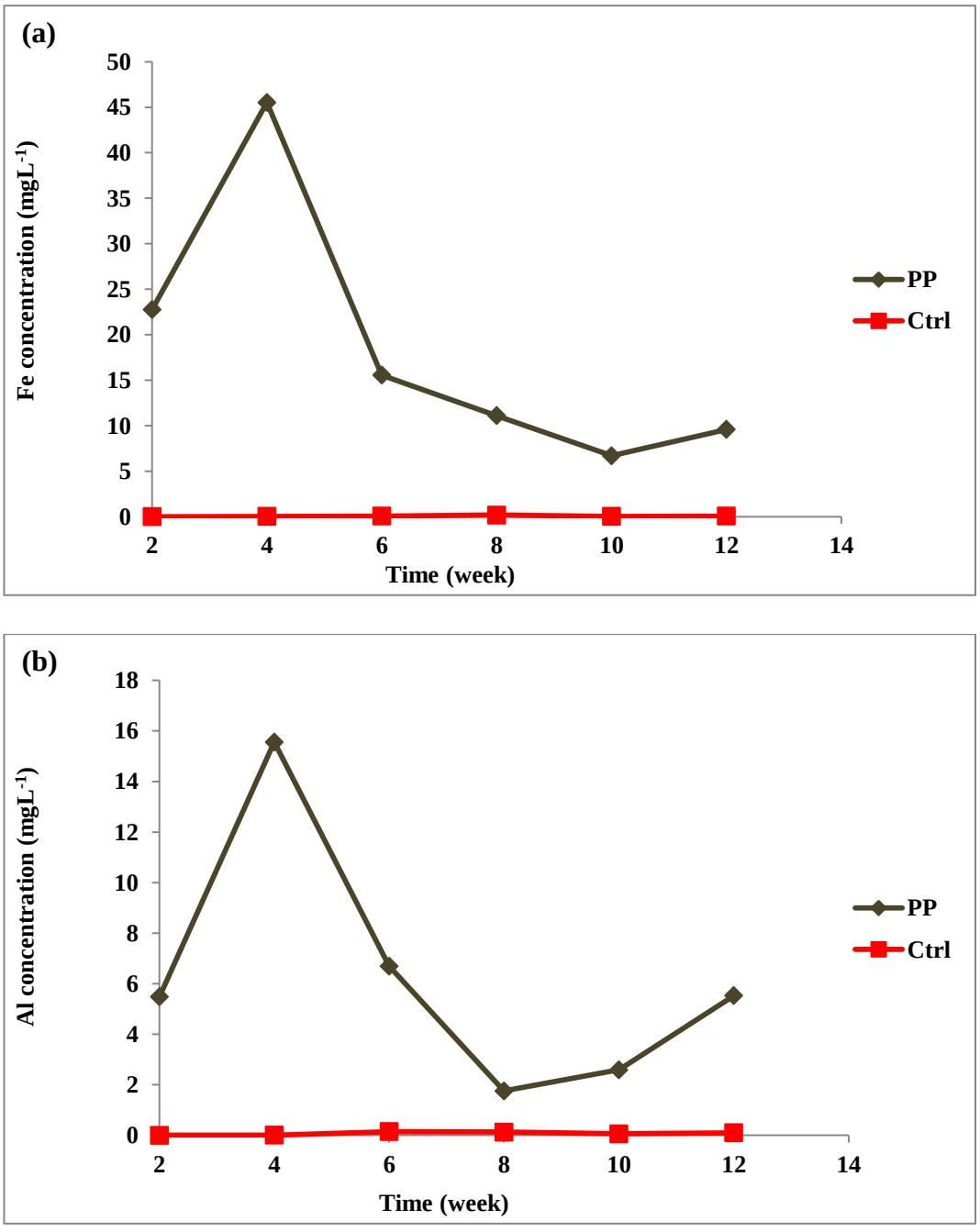

Figure 4.16: Effect of potato peel on concentration of (a) Fe and (b) Al in leachates as a function of time. Each point represents the average of three replicates.

The addition of potato peel amendments also led to a dramatic increase of the heavy metals in the leachates during the whole experiment. It indicates that the heavy metals inside the amended soil probably did not co-precipitate with Fe and Al.

One explanation for the observed increase in the metal leaching in potato peel amended soil might be that some components present in potato peel (polysaccharides, polyphenols, glycoalkaloides) can form complexes with the metals which are highly soluble in water. The formed highly soluble organometallic complexes can be easily drained due to water irrigation of the soil.

4.4.4. Effect of coconut husk amendments on metal concentrations in leachates

The effect of coconut husk amendment on Pb leaching can be deduced from Figure 4.17. The results show that coconut husk significantly ($P < 0.05$) increased Pb leaching during the period of the experiment compared to that of the control. The amendment specifically increased Pb leaching in Week 2 by 1 time, Week 4 by 3 times, Week 6 by 5 times, Week 8 by 6 times, Week 10 by 6 times and Week 12 by 5 times, compared to those of the control treatment.

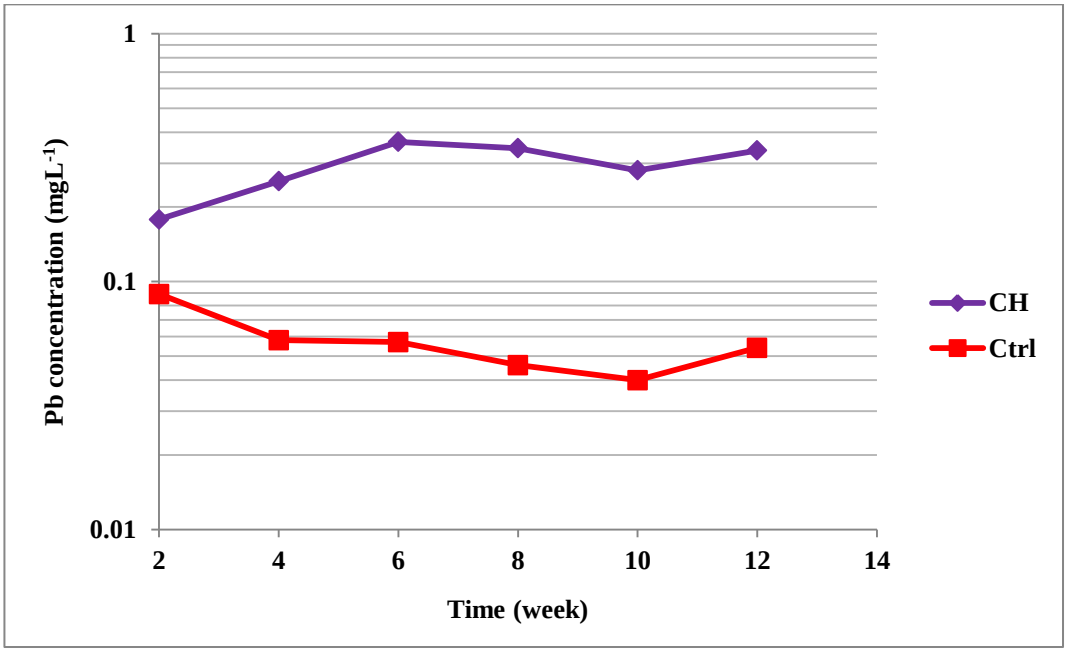

Figure 4.17: Effect of coconut husk on concentration of Pb in leachates as a function of time. Each point represents the average of three replicates.

The effect of coconut husk amendment on the concentrations of Cd in the leachates in the 12-week experiment is shown in Figure 4.18. Although Cd leaching in the amended soil had a decreasing trend, its concentrations were significantly ($P <$

0.0.5) higher than the control treatment during the whole experiment. Particularly, coconut husk amendment increased Cd concentrations in the leachates by 19% at Week 2, 147% at Week 4, 263% at Week 6, 387% at Week 8, 246% at Week 10 and 47% at Week 12, as compared to those of the control treatment.

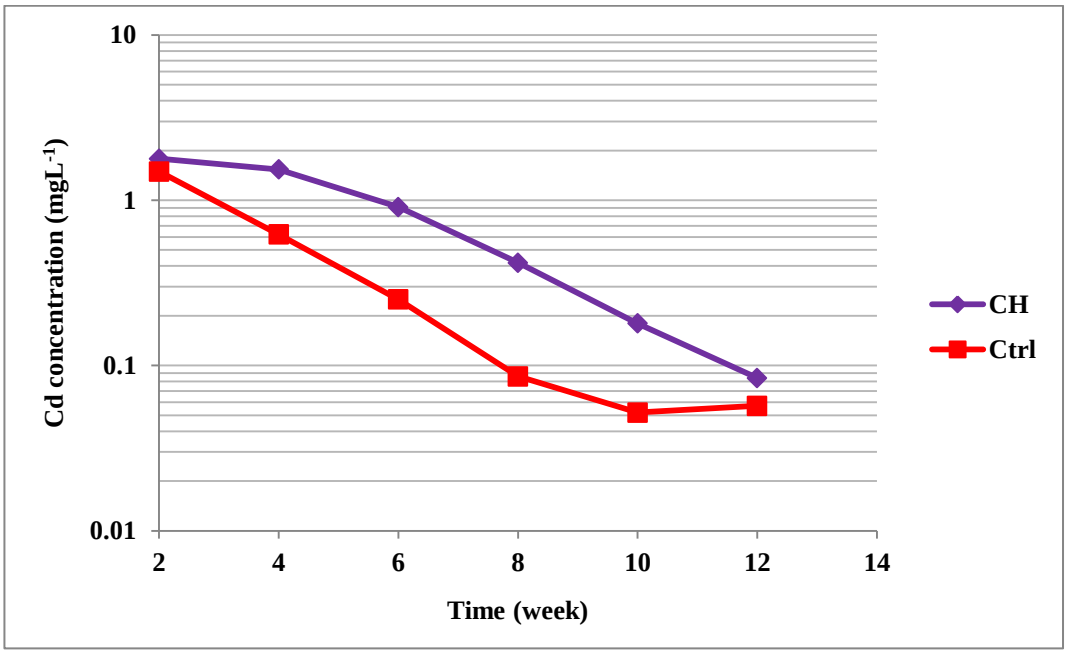

Figure 4.18: Effect of coconut husk on concentration of Cd in leachates as a function of time. Each point represents the average of three replicate.

The effect of coconut husk amendment on Zn leaching is presented in Figure 4.19. Zn leaching had a similar behavior to Cd leaching. Compared to the control, coconut husk significantly ($P < 0.0.5$) increased Zn leaching to 351% at Week 2, 871% at Week 4, 955% at Week 6, 994% at Week 8, 1000% at Week 10 and 432% at Week 12.

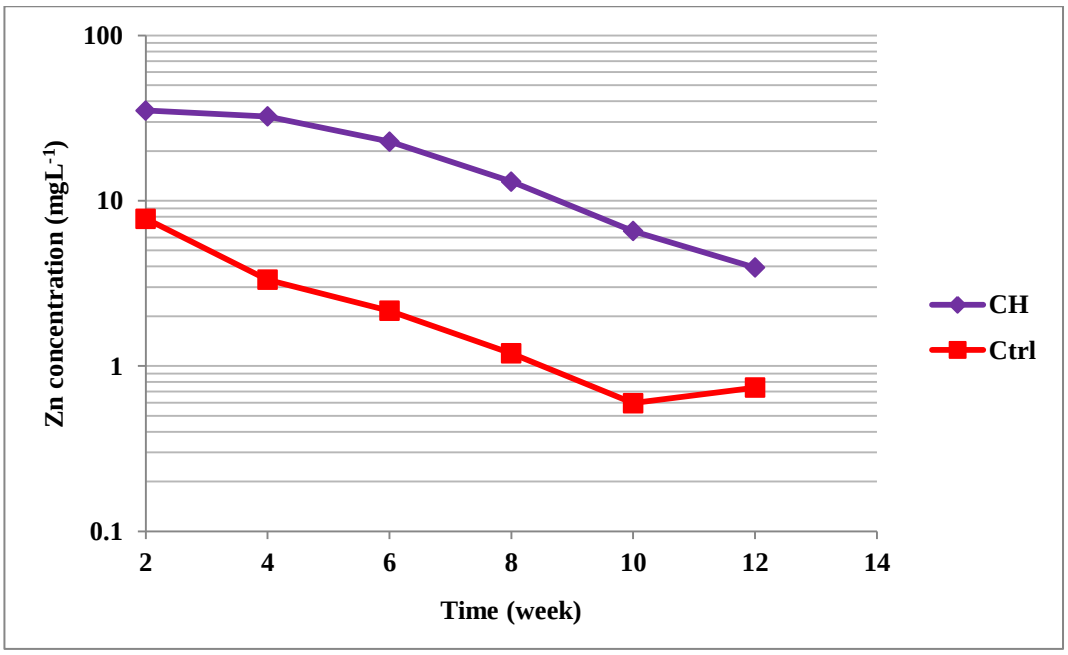

Figure 4.19: Effect of coconut husk on concentration of Zn in leachates as a function of time. Each point represents the average of three replicates.

Figure 4.20 illustrates the effect of coconut husk amendment on Cu leaching. Coconut husk significantly ($P < 0.05$) increased the concentrations of the Cu in the leachates from Week 4 until the end of the experiment, compared to those of the control. Cu leaching was increased by 25%, 107%, 207%, 234%, 309% and 463% at Week 2 until Week 12, respectively.

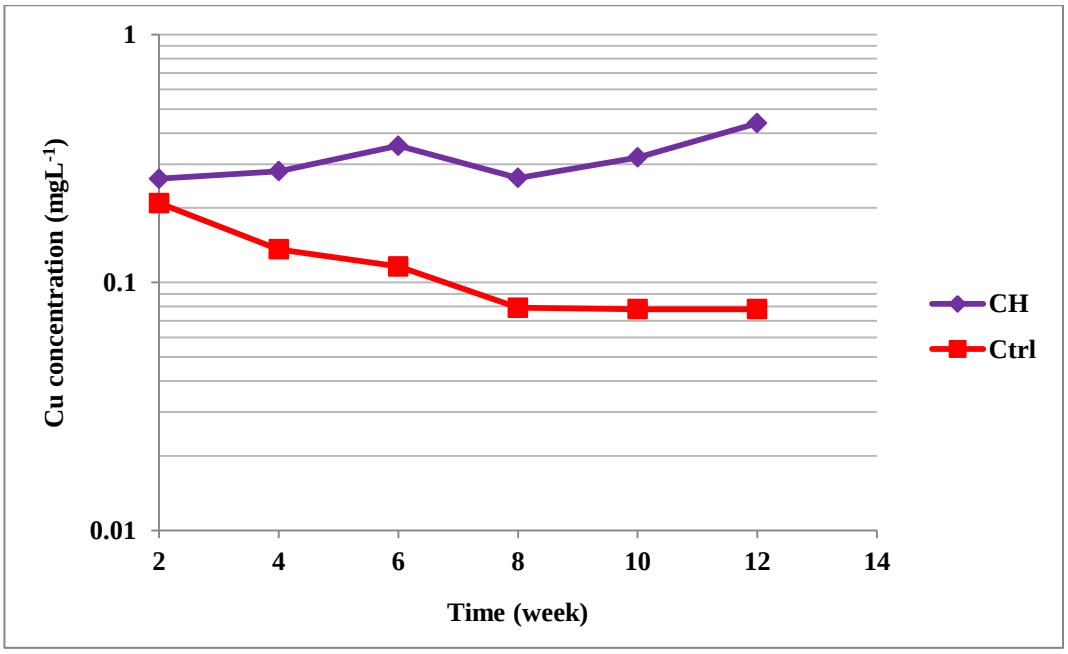

Figure 4.20: Effect of coconut husk on concentration of Cu in leachates as a function of time. Each point represents the average of three replicates.

Generally, in coconut husk amended soil, Pb and Cu exhibited a very similar leaching pattern while Cd and Zn showed almost same leaching behaviour. Similar to potato peel amendment, coconut husk amendment increased the leaching of all heavy metals including Fe and Al (Figure 4.21). Therefore, it is believed that the co-precipitation of Fe and/or Al hydroxides did not take place. Although the effect of the coconut husk amendment for the biosorption removal of heavy metals from aqueous wastes have been reported (Anirudhan & Shibi, 2007), this low-cost amendment was not effective for the immobilization of Pb, Cd, Zn and Cu in this specific type of the soil.

It is also well known that metals are less bioavailable in the soil with the pH of neutral to alkaline (Chen *et al.*, 2000; Hodson *et al.*, 2001; Zhao & Masaihiko, 2007). In this experiment, the addition of coconut husk amendment decreased the pH of leachate to pH 6.4 and this may explain why this amendment induced a great increase in the metal leaching.

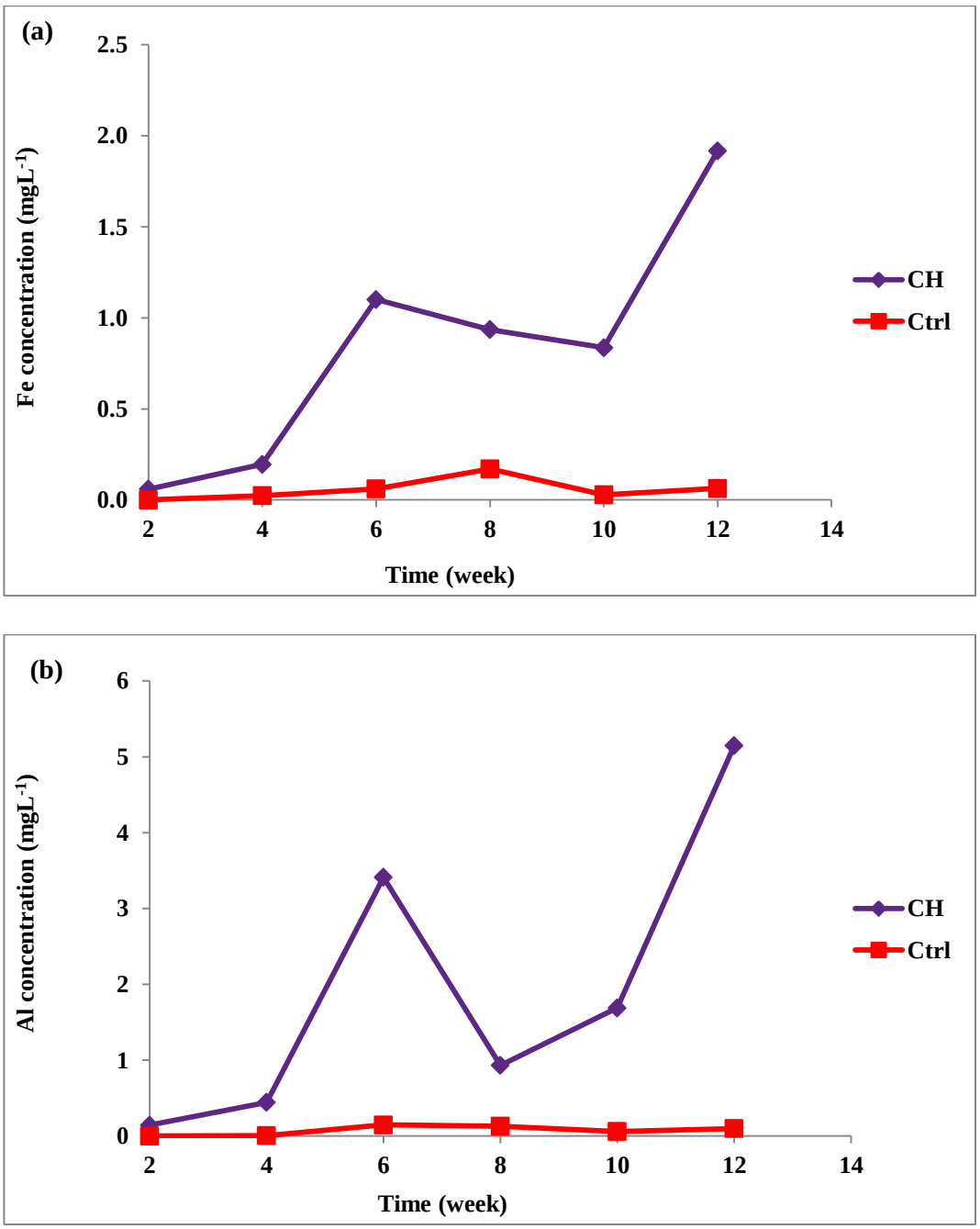

Figure 4.21: Effect of coconut husk on concentration of (a) Fe and (b) Al in leachates as a function of time. Each point represents the average of three replicates.

4.5. Effect of amendments on heavy metal leaching rate

The heavy metal leaching rate in the amended soils was calculated based on the amount of water added to the pot within the two weeks interval.

Generally, among the four amendments, potato peel amendment induced the highest Pb leaching rate whereas eggshell amendment caused the lowest Pb leaching rate (Table 4.3). The results from the metal leaching analysis indicated that regardless of the positive or negative effects of eggshell, banana stem and potato peel amendments, the concentration of Pb in the leachates decreased with time. This might be ascribed to the draining of highly soluble forms of Pb at the beginning of the experiment (Houben *et al.*, 2012). Thus, Pb leaching was less pronounced at the end of the experiment. However, in coconut husk amended soil the pattern of Pb leaching rate was different and it increased over the period of the experiment. The adsorption of Pb depends on various parameters such as pH or type of adsorbent. The lower soil pH contributes to lower Pb sorption in soil (Park *et al.*, 2011). Therefore, the increase in Pb leaching rate in coconut husk amended soil might be ascribed due to the acidic effect of the amendment arisen from the decomposition of organic matter content of coconut husk amendment. Specifically, the highest Pb leaching rate in coconut husk amended soil was 29.28 mg/day at Week 6 when the leachate was its lowest value pH, 6.5. (Table 4.3, Figure 4.1).

The highest Cd leaching rate was at the beginning of the experiment in all amended soils. It decreased remarkably during the 90 days experiment due to the drain of highly soluble forms of Cd (Table 4.3). The Cd leaching rate followed the order of coconut husk > potato peel > control > banana stem > eggshell.

Similar to the pattern of Cd leaching rate, Zn leaching rate followed the order of coconut husk > potato peel > control > banana stem > eggshell. The Zn leaching also had a decreasing pattern from Week 2 till Week 10 of the experiment (Table 4.3).

The behavior of Cu leaching rate in all amended soils was similar to Pb leaching rate where the highest leaching belongs to the potato peel amended soil followed by banana stem, coconut husk and eggshell amendments. Cu leaching in coconut husk amended soil, in contrast to other amendments, increased during the period of the experiment (Table 4.3). High concentration of organic matter of coconut husk amendment which could form highly soluble Cu-organic complexes might be the reason for high Cu leaching rate.

Table 4.3: Heavy metal leaching rate (mg/day) in the amended soils

Metal	Amendment	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Pb	EG	6.4	6.4	2.8	3.9	2.0	3.1
	BS	63.9	194.8	37.5	39.4	29.8	37.0
	PP	1472.0	2520.3	1042.1	735.0	517.8	578.3
	CH	14.2	20.3	29.3	27.6	22.5	27.0
	Ctrl	7.1	4.6	4.6	3.7	3.2	4.3
Cd	EG	21.7	7.3	3.1	1.2	0.5	0.9
	BS	7.9	21.5	6.2	3.4	2.9	3.3
	PP	56.0	123.4	45.3	34.2	27.4	30.4
	CH	142.7	123.0	72.8	33.5	14.4	6.7
	Ctrl	119.4	49.7	20.1	6.9	4.2	4.6
Zn	EG	183.9	73.7	54.3	34.7	9.2	20.7
	BS	149.8	356.0	92.7	78.3	62.2	82.1
	PP	1868.0	3732.0	1229.6	836.6	650.4	826.3
	CH	2808.5	2587.2	1823.7	1043.4	524.7	315.3
	Ctrl	622.2	266.3	172.8	95.4	47.7	59.3
Cu	EG	27.3	13.8	13.4	8.1	8.4	7.8
	BS	132.7	305.1	79.7	58.3	54.9	62.5
	PP	3748.0	6442.7	2955.7	1829.3	1493.9	1300.5
	CH	21.0	22.5	28.5	21.1	25.5	35.1
	Ctrl	16.7	10.9	9.3	6.3	6.2	6.2

EG: Eggshell, BS: Banana Stem, PP: Potato Peel, CH: Coconut Husk, Ctrl: Control

4.6. Heavy metal distributions and fractionation in soil

The metal leaching analysis yielded information about the total concentration of a heavy metal percolated into the leachates. However, it only provides some indication of contamination level in the soil and may not be very informative in terms of potential environmental and human health risks. Therefore, the forms of the heavy metals in the soil induced by the addition of the amendments were determined by the sequential extraction analysis.

From the results obtained in the metal leaching analysis, it was found that two amendments, eggshell and banana stem, were remarkably effective on the reduction of some heavy metals whereas potato peel and coconut husk amendments did not have any positive effect on the metal reduction in the leachates. Therefore, the sequential extraction analysis was carried out for eggshell and banana stem treated soils along with control treatment.

The distributions and percentages of Pb, Cd, Zn and Cu chemical fractions in eggshell and banana stem amended soils and in control treatment are shown in Figures 4.22 - 4.25 and the results from the sequential extraction analysis are discussed in the following section.

4.6.1. Effect of amendments on Pb fractionation in soil

Most of Pb in both amended soils and control treatment was associated with carbonate fraction (Figure 4.22). The addition of eggshell as amendment significantly ($P < 0.05$) decreased the concentration of Pb in exchangeable fraction from 11.3% to 3.7%, and increased it remarkably in residual fraction from 18.5% to 27.6%. The addition of banana stem amendment also altered Pb fractions distribution in the soil. It increased Pb

concentrations significantly ($P < 0.05$) in fractions bound to organic matter from 0.7% to 5.4%, and significantly ($P < 0.05$) reduced Pb concentrations in both exchangeable and residual fractions in the soil from 11.3% to 5.4% and from 18.5% to 5%, respectively.

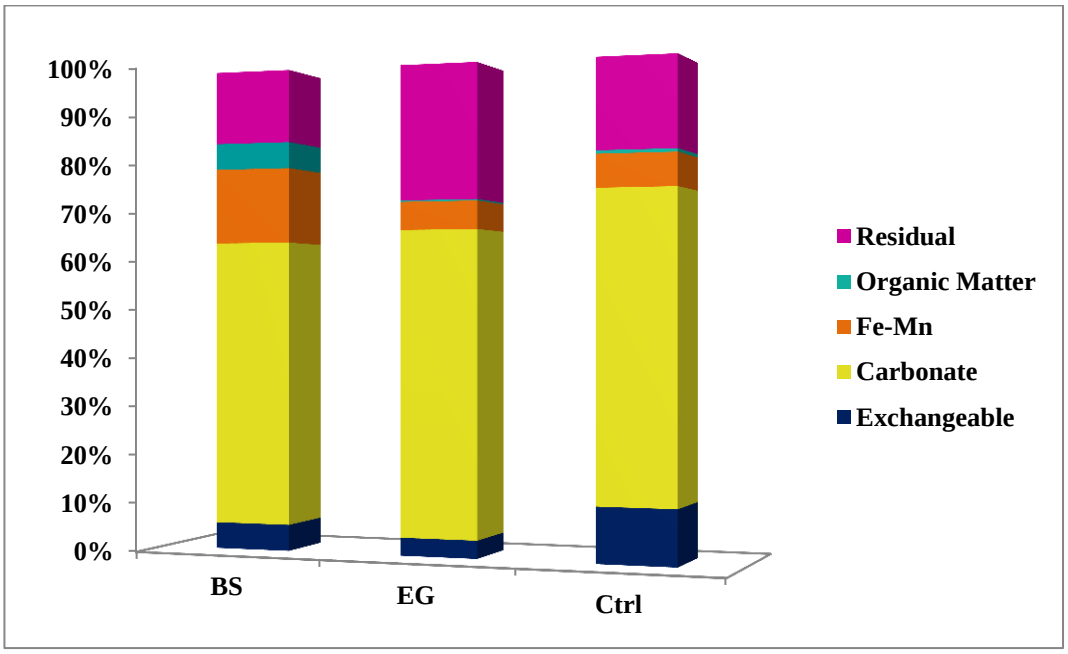

Figure 4.22: Pb distribution and fractionation by sequential extraction in the control treatment (Ctrl), eggshell amended soil (EG) and banana stem amended soil (BS).

The heavy metals in residual fraction mainly are within their crystal structure (Tessier *et al.*, 1979). Thus, they are not expected to be released under natural conditions over a specific period of time and as a result, environmental risk of the metals in this fraction would be much lesser than other forms of metals. The reduction of Pb in residual form in banana stem treated soil may be considered as the cause for the high Pb leaching in this amended soil (Table 4.3) while the increase in residual Pb fraction induced by eggshell amendment reflecting Pb immobilization to, and within, the inorganic surface. Pb in eggshell amended soil is held to the surface of eggshell due to the high residual surface alkalinity and high surface pH of the amendment (Zhou *et al.*, 2012).

4.6.2. Effect of amendments on Cd fractionation in soil

Despite the differences in fraction percentages in the amended soils and control treatment, the distributions of Cd in the five chemical fractions exhibited a similar patterns (Residual > Exchangeable > Carbonate > Fe-Mn > Organic Matter) (Figure 4.23). The addition of eggshell or banana stem amendments altered exchangeable and residual fractions of Cd in the soils remarkably. Moreover, residual fraction of Cd was increased in both eggshell and banana stem treated soils from 59.7% to 64.3% and 74.4%, respectively. Therefore, both amendments were effective in the immobilization of Cd in the soils. This is in agreement with the results obtained from Cd leaching rate where eggshell and banana stem amended soils had the lower Cd leaching rate compared to other amendments and control (Table 4.3).

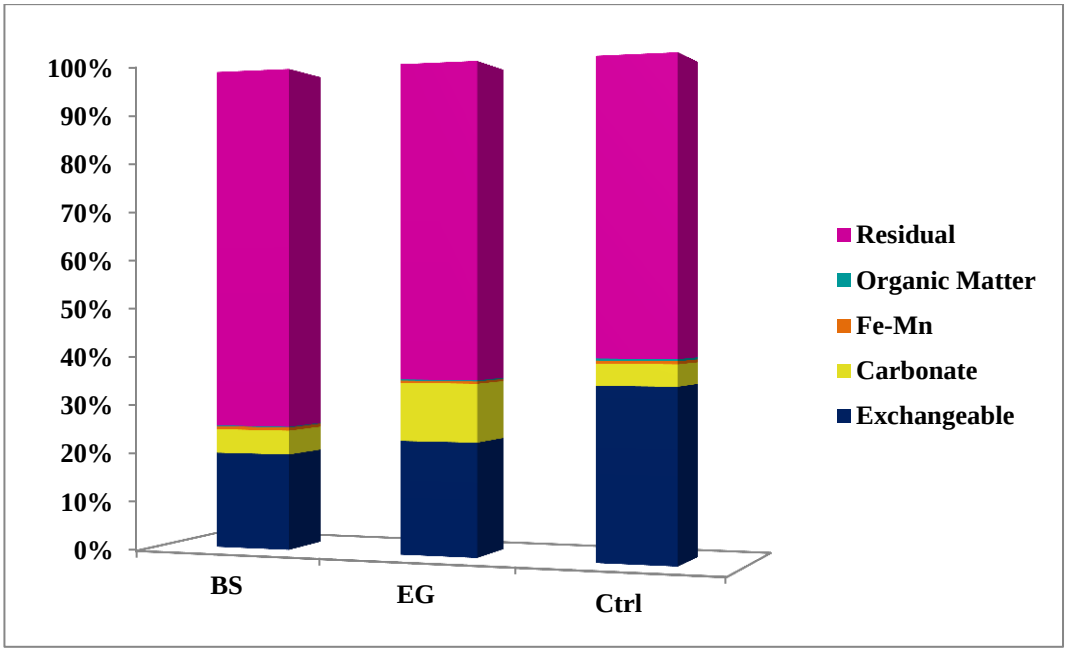

Figure 4.23: Cd distribution and fractionation by sequential extraction in the control treatment (Ctrl), eggshell amended soil (EG) and banana stem amended soil (BS).

Since metals associated with residual fraction in the soil are less likely to be released to the leachates through dissociation (Nemati *et al.*, 2011), the conversion of labile Cd to non-mobile Cd form supports the low concentration of Cd in the leachates of both amended soils. The sequential extraction results also show a significant ($P <$

0.05) increase in bound to carbonate fraction of Cd in eggshell amended soil. This might be due to the high $CaCO_3$ content in eggshell which promotes the formation of $CdCO_3$.

4.6.3. Effect of amendments on Zn fractionation in soil

The distribution of Zn fractions in both amended soils and control treatment was similar and followed the order Carbonate > Residual > Exchangeable > Fe-Mn > Organic Matter (Figure 4.24). Compared to control treatment, the application of eggshell amendment significantly ($P < 0.05$) reduced exchangeable fraction of Zn. Moreover, eggshell amendment had slightly increased residual fraction of Zn in the soil from 10.2% to 12.5%, whereas residual form of Zn decreased in the treatment with banana stem amendment from 10.2% to 10.1%. Zn concentration in fraction bound to carbonate in eggshell amended soil increased slightly compared to that of control treatment (from 68.8% to 74.5%) which might attributed to the formation of $ZnCO_3$ in the amended soil.

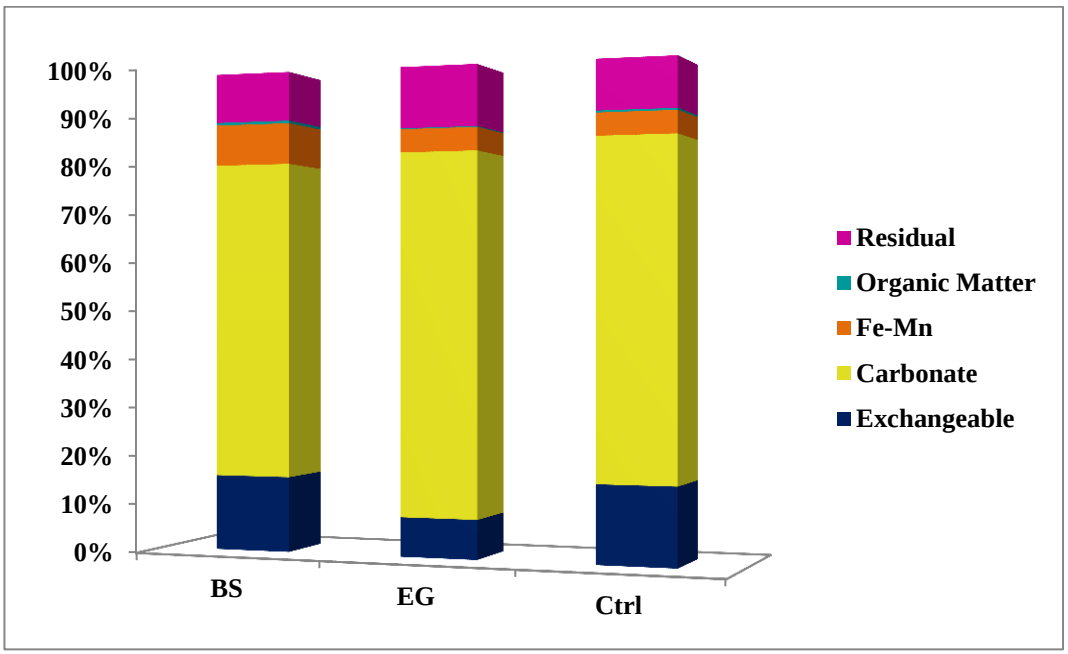

Figure 4.24: Zn distribution and fractionation by sequential extraction in the control treatment (Ctrl), eggshell amended soil (EG) and banana stem amended soil (BS).

Exchangeable fraction includes those metals which are adsorbed to the solid surface by relatively weak electrostatic interaction (Filgueiras *et al.*, 2002). Hence, they can be easily removed from the soil structure through sorption-desorption processes. In this experiment, exchangeable fraction of Zn was reduced by eggshell amendment while it was increased by banana stem amendment. These results are in agreement with the results obtained from the metal leaching analysis where Zn leaching was reduced in eggshell treated soil which could be attributed to the reduction of exchangeable form of Zn. The reduction of Zn in residual form in banana stem treated soil may affirm its high leaching at Week 12 of the experiment (Table 4.3).

4.6.4. Effect of amendments on Cu fractionation in soil

The fractionations of Cu in the amended and control soils are presented in Figure 4.25. The results shows that copper contents in eggshell treated soil and control treatment, through sequential extraction fractionation are in the order of Carbonate > Fe-Mn > Residual > Organic Matter > Exchangeable, while Cu content in banana stem treated soil is in the order of Carbonate > Fe-Mn > Organic Matter > Residual > Exchangeable. The dominant forms of Cu in all treatment were bound to carbonate followed by the binding to Fe-Mn oxides. The addition of eggshell amendment had significantly ($P < 0.05$) decreased the Cu concentration in exchangeable fraction from 2.7% to 1.9%. Banana stem amendment significantly ($P < 0.05$) decreased carbonate fraction of Cu in the soil from 76.1% to 31.8%, and significantly ($P < 0.05$) increased Cu concentration in the fraction bound to organic matters from 2.9% to 20%. These results do not support the results from the metal leaching analysis. However, it is assumed that the results from the metal leaching are more reliable as it is the result collected from 90-days experiment. Therefore, control treatment had the lowest Cu leaching rate compared to the amended soils indicating the negative effect of the amendments on Cu immobilization in the soil.

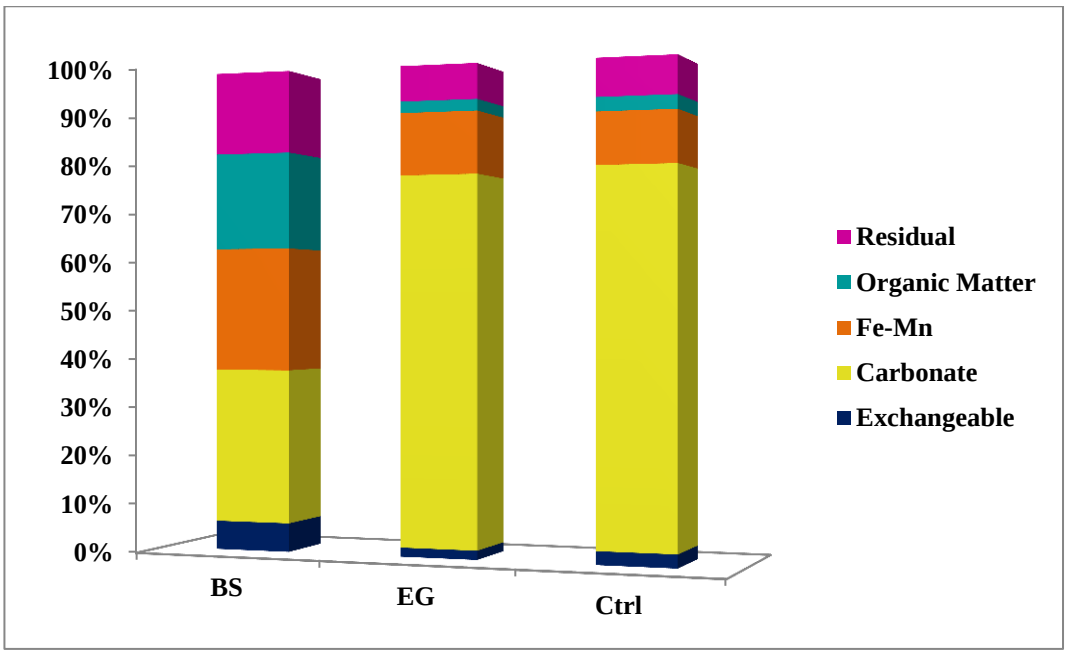

Figure 4.25: distribution and fractionation by sequential extraction in the control treatment (Ctrl), eggshell amended soil (EG) and banana stem amended soil (BS).

4.7. Contamination Factor (CF) of heavy metals

According to Nemati *et al*. (2011), the contamination factor of a heavy metal indicates the degree that the metal can pose risks to the environment in respect to its retention time (Nemati *et al.*, 2011). Hence, a high contamination factor of a heavy metal shows a low retention time and a high risk to the environment. Figure 4.26 shows the estimated contamination factor of each heavy metal in eggshell and banana stem amended soils and control treatment.

The individual contamination factor (CF) of the heavy metals was determined using the equation below:

Contamination factor (CF) = sum of each heavy metal concentration in the mobile phase (non-residual phase) / its concentration in the residual phase

Since the contamination factor is calculated based on the sequential extraction procedures, the contamination factor of Pb, Cd and Zn are discussed here.

The calculated contamination factor shows that the environmental risks of Pb, Cd and Zn in both amended soils and control treatment are in the order of Zn > Pb > Cd. These results were expectable because as mentioned before, most of Cd in both amended soils and control treatment was in residual form. Residual concentration of heavy metals is considered as the non-mobile phase and it is very important because it influences the mobility of the heavy metals. Most of Pb and Zn were associated with carbonate fraction. However, residual fraction of Pb in both amended soils and control treatment were moderately higher than that of Zn in all treatments which probably resulted in lower contamination factor.

Generally, the lowest contamination factor of Cd was induced by banana stem amendment followed by eggshell amendment. On contrary, the lowest contamination factors of Pb and Zn were observed in eggshell amended soil indicating the high potential ability of this amendment to fix these heavy metals in the soil which can be attributed to its high Ca content. Thus, the risks of these metals in ecosystem such as their toxicity to animals, plants and microorganisms are diminished.

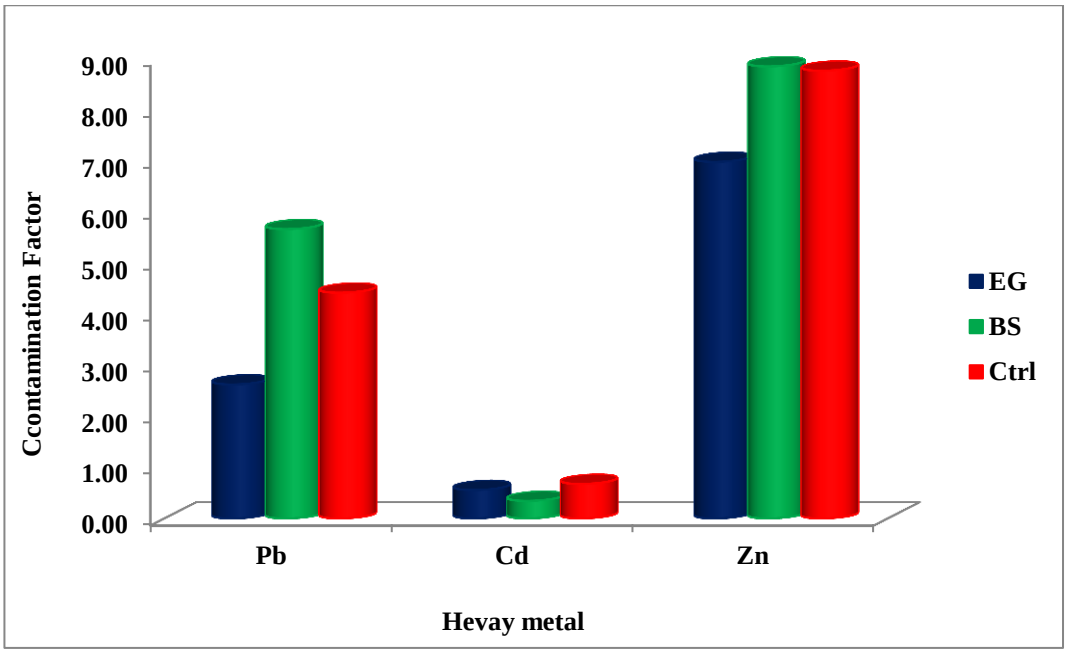

Figure 4.26: Contamination factor in the eggshell (EG), banana stem (BS) amended soils and the control treatment (Ctrl).

CHAPTER 5

CONCLUSIONS

The results from the metal leaching analysis indicated that the addition of eggshell and banana stem amendments effectively decreased the concentration of Cd in the leachates. Moreover, eggshell amendment reduced the concentrations of Zn in the leachates remarkably and to a lesser extent the concentrations of Pb in the leachates. The addition of potato peel and coconut husk amendments did not have any positive effect on heavy metal immobilization but also increased greatly the concentrations of the heavy metals in leachates. Our study implies that eggshell amendment can be used as a low-cost and environmentally safe additive for the *in situ* immobilization of Pb, Cd and Zn in a sandy soil with the pH of slightly alkaline. Banana stem amendment can also be applied as a cost-effective fixing amendment in a Cd-contaminated soil in this specific type of the soil. The results from the sequential extraction analysis showed that eggshell amendment reduced exchangeable fraction of Pb, Cd and Zn in the soil and increased residual fraction which is the most stable form of metals in the soil. Banana stem amendment also reduced exchangeable form of Cd in the soil and transformed it into residual fraction which resulted in lowering the Cd mobility in the amended soil. The use of different immobilizing amendments have a different impact on the metal (im)mobilization; hence, the effect of each amendment is case specific and any generalizations are not appropriate to make. Overall, this study concludes that among all four amendments, eggshell amendment is found to be the most effective fixing additive for *in situ* immobilization of Pb, Cd and Zn in the soil due to the its high $CaCO_3$.

References

Ambient Air Quality Standards (AAQS) for Particulate Matter. (2014). *California Environmental Protection Agency, Air Resources Board*. Retrieved April 2013, from http://www.arb.ca.gov/research/aaqs/pm/pm.htm

Abaidoo, R. C., Keraita, B., Drechsel, P., Dissanayake, P., & Maxwell, A. S. (2010). Soil and crop contamination through wastewater irrigation and options for risk reduction in developing countries. In *Soil Biology and Agriculture in the Tropics*, 275-297. Berlin Heidelberg: Springer.

Adriano, D., Wenzel, W., Vangronsveld, J., & Bolan, N. S. (2004). Role of assisted natural remediation in environmental cleanup. *Geoderma*, 122(2), 121-142.

Adriano, D. C. (2001). Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals. New York, USA: Springer.

Ahmad, M., Hashimoto, Y., Moon, D. H., Lee, S. S., & Ok, Y. S. (2012). Immobilization of lead in a Korean military shooting range soil using eggshell waste: an integrated mechanistic approach. *Journal of hazardous materials*, 209: 392-401.

Al-Weshahy, A., & Rao, V. (2012). Potato Peel as a Source of Important Phytochemical Antioxidant Nutraceuticals and Their Role in Human Health–A Review. In Rao. V. (Eds.), *Phytochemicals as nutraceuticals–global approaches to their role in nutrition and health*, 207. Rijeka, Croatia: InTech

Anirudhan, T., & Shibi, I. (2007). Preparation of a cation exchanger containing carboxyl groups from banana stalk and its utilization as chelating agent. *Infomusa*, 16(1&2), 7-11.

Bioavailability of contaminants in soils and sediments: processes, tools, and applications. (2003). National Research Council (NRC), Washington, D.C.: National Academies Press.

Bolan, N. S., Adriano, D. C., & Naidu, R. (2003). Role of phosphorus in (im) mobilization and bioavailability of heavy metals in the soil-plant system. *Reviews of Environmental Contamination and Toxicology*, 177: 1-44. New York, USA: *Springer*.

Bolan, N. S., Naidu, R., Syers, J. K., & Tillman, R. (1999). Surface charge and solute interactions in soils. *Advances in agronomy*, 67: 87-140.

Campbell, P. G. (2007). Cadmium_a priority pollutant. *Environmental Chemistry*, 3(6), 387-388.

Canet, R., Pomares, F., Tarazona, F., & Estela, M. (1998). Sequential fractionation and plant availability of heavy metals as affected by sewage sludge applications to soil. *Communications in Soil Science & Plant Analysis*, 29(5&6), 697-716.

Chandrajith, R., & Dissanayake, C. (2009). Phosphate mineral fertilizers, trace metals and human health. *Journal of the National Science Foundation of Sri Lanka*, 37(3), 153-165.

Chen, H., Zheng, C., Tu, C., & Shen, Z. (2000). Chemical methods and phytoremediation of soil contaminated with heavy metals. *Chemosphere*, 41(1), 229-234.

Chu, W., & Chan, K. (2003). The mechanism of the surfactant-aided soil washing system for hydrophobic and partial hydrophobic organics. *Science of the Total Environment*, 307(1), 83-92.

Copper and compounds (Fact sheet). (2014). *Australian Government, Department of the Environment and Heritage*. Retrieved April 2013, from http://www.npi.gov.au/resource/copper-and-compounds

Darvodelsky, P., & Bridle, T. (2012). Biosolids Snapshot. *Improving our environment Pollution Solutions and Designs Pty Ltd*. Retrieved September 2013, from http://www.environment.gov.au/system/files/resources/2e8c76c3-0688-47ef-a425-5c89dffc9e04/files/biosolids-snapshot.pdf.

de la Fuente, C., Clemente, R., Martínez-Alcalá, I., Tortosa, G., & Bernal, M. P. (2011). Impact of fresh and composted solid olive husk and their water-soluble fractions on soil heavy metal fractionation; microbial biomass and plant uptake. *Journal of hazardous materials,* 186(2), 1283-1289.

Diels, L., Van der Lelie, N., & Bastiaens, L. (2002). New developments in treatment of heavy metal contaminated soils. *Reviews in Environmental Science and Biotechnology,* 1(1), 75-82.

Domańska, J. (2008). Sequential fractionation of lead in contaminated and non-contaminated soils. *Polish Journal of Soil Science*, 41: 119-126.

Doumett, S., Lamperi, L., Checchini, L., Azzarello, E., Mugnai, S., Mancuso, S., . . . Del Bubba, M. (2008). Heavy metal distribution between contaminated soil and Paulownia tomentosa, in a pilot-scale assisted phytoremediation study: influence of different complexing agents. *Chemosphere,* 72(10), 1481-1490.

Drechsel, P., Scott, C. A., Raschid-Sally, L., Redwood, M., & Bahri, A. (2009). Wastewater irrigation and health. *Assessing and Mitigating Risk in Low-income Countries. London: Earthscan*.

Dutch Target and Intervention Values (the New Dutch List). (2000). *Esdat Environmental Database Management Software.* Retrieved December 2013, from http://www.esdat.net/Environmental%20Standards/Dutch/annexS_I2000Dutch%20Environmental%20Standards.pdf

EPA's Air and radiation – Air pollution: Lead in air. (2012). *U.S. Environmental Protection Agency.* Retrieved April 2013, from http://www.epa.gov/air/lead/

EPA's Air and radiation – Cadmium compounds. (2013). *U.S. Environmental Protection Agency.* Retrieved April 2013, from http://www.epa.gov/ttnatw01/hlthef/cadmium.html

EPA'S Coppers Facts. (2008). *U.S. Environmental Protection Agency*. Retrieved April 2013, from http://www.epa.gov/oppsrrd1/REDs/factsheets/copper_red_fs.pdf

EPA's Engineering Bulletin Soil Washing Treatment. (1990). *U.S. Environmental Protection Agency*. Retrieved April 2013, from http://www.epa.gov/nscep/index.html

EPA's Guide for Industrial Waste Management. (1999). *U.S. Environmental Protection Agency*. Retrieved April 2013, from http://www.csu.edu/cerc/researchreports/documents/EPAGuideForIndustrialWasteManagement2002.pdf

EPA's Method 6020A. (2007). *U.S. Environmental Protection Agency*. Retrieved September 2013, from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/6020a.pdf

EPA's National Hardrock Mining Framework – Appendix B: Potential Environmental Impacts of Hardrock Mining. (1997). *U.S. Environmental Protection Agency Office of Water (4203) 401 M Street, SW Washington, DC 20460*. Retrieved April 2013, from http://www.epa.gov/aml/policy/app_b.pdf

EPA's Recent Developments for *In situ* Treatment of Metal Contaminated Soils. (1997). *U.S. Environmental Protection Agency, Solid Waste and Emergency Response (20460) Washington D.C.* Retrieved April 2013, from http://epa.gov/tio/download/remed/metals2.pdf

EPA's Toxicological Review of Zinc and Compounds. (2005). *U.S. Environmental Protection Agency.* Retrieved April 2013, from http://www.epa.gov/iris/toxreviews/0426tr.pdf

EPA'S Treatment Technologies for Site Cleanup: Twelfth Edition. (2007). *U.S. Environmental Protection Agency- Annual Status Report*. Retrieved April 2013, from http://epa.gov/tio/download/remed/asr/12/asr12_full_document.pdf

Evanko, C. R., & Dzombak, D. A. (1997). *Remediation of metals-contaminated soils and groundwater*. Pittsburgh, PA: Ground-water remediation technologies analysis center.

Fan, J., He, Z., Ma, L. Q., Yang, Y., Yang, X., & Stoffella, P. J. (2011). Immobilization of copper in contaminated sandy soils using calcium water treatment residue. *Journal of hazardous materials*, 189(3), 710-718.

Farrell, M., Perkins, W. T., Hobbs, P. J., Griffith, G. W., & Jones, D. L. (2010). Migration of heavy metals in soil as influenced by compost amendments. *Environmental Pollution*, 158(1), 55-64.

Feng, D., Aldrich, C., & Tan, H. (2000). Treatment of acid mine water by use of heavy metal precipitation and ion exchange. *Minerals Engineering*, 13(6), 623-642.

Filgueiras, A. V., Lavilla, I., & Bendicho, C. (2002). Chemical sequential extraction for metal partitioning in environmental solid samples. *Journal of Environmental Monitoring*, 4(6), 823-857.

Fitzgerald, D. J. (1998). Safety guidelines for copper in water. *The American journal of clinical nutrition*, 67(5), 1098S-1102S.

Gadepalle, V. P., Ouki, S. K., Herwijnen, R. V., & Hutchings, T. (2007). Immobilization of heavy metals in soil using natural and waste materials for vegetation establishment on contaminated sites. *Soil & Sediment Contamination*, 16(2), 233-251.

Gao, Y., He, J., Ling, W., Hu, H., & Liu, F. (2003). Effects of organic acids on copper and cadmium desorption from contaminated soils. *Environment International*, 29(5), 613-618.

Gosar, M. (2004). Environmental impacts of metal mining = Vplivi kovinskih rudnikov na okolje. *RMZ-Materials and geoenvironment*, 51(4), 2097-2107.

Guo, G., Zhou, Q., & Ma, L. Q. (2006). Availability and assessment of fixing additives for the in situ remediation of heavy metal contaminated soils: a review. *Environmental Monitoring and Assessment*, 116(1-3), 513-528.

Hale, B., Evans, L., & Lambert, R. (2012). Effects of cement or lime on Cd, Co, Cu, Ni, Pb, Sb and Zn mobility in field-contaminated and aged soils. *Journal of hazardous materials*, 199: 119-127.

Hamilton, A. J., Stagnitti, F., Xiong, X., Kreidl, S. L., Benke, K. K., & Maher, P. (2007). Wastewater irrigation: The state of play. *Vadose Zone Journal*, 6(4), 823-840.

Harter, R. D., & Naidu, R. (1995). Role of metal-organic complexation in metal sorption by soils. *Advances in agronomy*, 55: 219-263.

Hashimoto, Y., Matsufuru, H., Takaoka, M., Tanida, H., & Sato, T. (2009). Impacts of chemical amendment and plant growth on lead speciation and enzyme activities in a shooting range soil: an X-ray absorption fine structure investigation. *Journal of environmental quality*, 38(4), 1420-1428.

Haynes, R., Murtaza, G., & Naidu, R. (2009). Inorganic and organic constituents and contaminants of biosolids: implications for land application. *Advances in agronomy*, 104: 165-267.

Hodson, M., Valsami-Jones, E., Cotter-Howells, J., Dubbin, W., Kemp, A., Thornton, I., & Warren, A. (2001). Effect of bone meal (calcium phosphate) amendments on metal release from contaminated soils_a leaching column study. *Environmental Pollution*, 112(2), 233-243.

Houben, D., Evrard, L., & Sonnet, P. (2013). Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. *Chemosphere*, 92(11), 1450-1457.

Houben, D., Pircar, J., & Sonnet, P. (2012). Heavy metal immobilization by cost-effective amendments in a contaminated soil: Effects on metal leaching and phytoavailability. *Journal of Geochemical Exploration*, 123: 87-94.

Houben, D., & Sonnet, P. (2010). Leaching and phytoavailability of zinc and cadmium in a contaminated soil treated with zero-valent iron. In *Proceedings of the 19th World Congress of soil science, soil solutions for a changing World* (1-6).

Huang, J.-H., Hsu, S.-H., & Wang, S.-L. (2011). Effects of rice straw ash amendment on Cu solubility and distribution in flooded rice paddy soils. *Journal of hazardous materials*, 186(2), 1801-1807.

Janoš, P., Vávrová, J., Herzogová, L., & Pilařová, V. (2010). Effects of inorganic and organic amendments on the mobility (leachability) of heavy metals in contaminated soil: a sequential extraction study. *Geoderma*, 159(3), 335-341.

Järup, L. (2003). Hazards of heavy metal contamination. *British medical bulletin*, 68(1), 167-182.

Jiménez, B. (2006). Irrigation in developing countries using wastewater. *International Review for Environmental Strategies*, 6(2), 229-250.

Jones, L., & Jarvis, S. (1981). The fate of heavy metals. In *The chemistry of soil processes.* Greenland, D. J. & M. H. B. Hayes (Eds.) 593-620. New York: Wiley.

Kabata-Pendias A. (2000). Trace elements in soils and plants. Boca Raton, FL, USA: CRC press.

Kent, D., Abrams, R., Davis, J., Coston, J., & LeBlanc, D. (2000). Modeling the influence of variable pH on the transport of zinc in a contaminated aquifer using semiempirical surface complexation models. *Water resources research*, 36(12), 3411-3425.

Khai, N. M., Tuan, P. T., Vinh, N. C., & Oborn, I. (2008) Effects of using wastewater as nutrient sources on soil chemical properties in peri-urban agricultural systems. *VNU Journal of Science, Earth Sciences*, 24: 87-95.

Khan, S., Cao, Q., Zheng, Y., Huang, Y., & Zhu, Y. (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution*, 152(3), 686-692.

Kumpiene, J., Lagerkvist, A., & Maurice, C. (2008). Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments–a review. *Waste management*, 28(1), 215-225.

Lasat, M. (2000). Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. *Journal of Hazardous Substance Research*, 2(5), 1-25.

Lead alert facts: Lead and the environment (Fact sheet). (n.d.). *Australian Government, Department of the Environment and Heritage*. Retrieved April 2013, from http://www.environment.gov.au/resource/lead-alert-facts-lead-and-environment

Lead Poisoning. (2009). *National Safety Council*. Retrieved April 2013, from http://www.nsc.org/news_resources/Resources/Documents/Lead_Poisoning.pdf

Lee, M., Paik, I. S., Kim, I., Kang, H., & Lee, S. (2007). Remediation of heavy metal contaminated groundwater originated from abandoned mine using lime and calcium carbonate. *Journal of hazardous materials*, 144(1), 208-214.

Lee, S.-H., Park, H., Koo, N., Hyun, S., & Hwang, A. (2011). Evaluation of the effectiveness of various amendments on trace metals stabilization by chemical and biological methods. *Journal of hazardous materials*, 188(1), 44-51.

Lee SS, Lim JE, El-Azeem SAMA, Choi B, Oh S-E, Moon DH, Ok YS. (2013). Heavy metal immobilization in soil near abandoned mines using eggshell waste and rapeseed residue. *Environmental Science and Pollution Research*, 20:1719-1726.

Li, K., Fu, S., Zhan, H., Zhan, Y., & Lucia, L. (2010). Analysis of The Chemical Composition and Morphological Structure of Banana Pseudo-Stem. *BioResources*, 5(2), 576-585.

Li, P., Wang, X., Zhang, T., Zhou, D., & He, Y. (2008). Effects of several amendments on rice growth and uptake of copper and cadmium from a contaminated soil. *Journal of Environmental Sciences*, 20(4), 449-455.

Ma, Q. Y., Traina, S. J., Logan, T. J., & Ryan, J. A. (1993). In situ lead immobilization by apatite. *Environmental Science & Technology*, 27(9), 1803-1810.

Ma, R., Zong, Y., & Lu, S. (2012). Reducing Bioavailability and Leachability of Copper in Soils using Coal Fly Ash, Apatite, and Bentonite. *Communications in Soil Science and Plant Analysis*, 43(15), 2004-2017.

Manahan, S. E. (2002). Toxicological chemistry and biochemistry. Boca Raton, FL, USA: CRC Press.

Martin, T. A., & Ruby, M. V. (2004). Review of in situ remediation technologies for lead, zinc, and cadmium in soil. *Remediation Journal*, 14(3), 35-53.

Martínez, C., & Motto, H. (2000). Solubility of lead, zinc and copper added to mineral soils. *Environmental Pollution*, 107(1), 153-158.

Metals _Copper. (2010). *Netherlands Environmental Assessment Agency (PBL)*. Retrieved April 2013, from http://themasites.pbl.nl/tridion/en/themasites/hyde/productiondata/metals/index-2.html

McLaughlin, M. J., Hamon, R., McLaren, R., Speir, T., & Rogers, S. (2000). Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Soil Research*, 38(6), 1037-1086.

Mulligan, C., Yong, R., & Gibbs, B. (2001). Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering geology*, 60(1), 193-207.

Nemati, K., Bakar, N. K. A., Abas, M. R., & Sobhanzadeh, E. (2011). Speciation of heavy metals by modified BCR sequential extraction procedure in different depths of sediments from Sungai Buloh, Selangor, Malaysia. *Journal of hazardous materials*, 192(1), 402-410.

Nilsson, J.-A., & Randhem, J. (2008). *Environmental Impacts and Health Aspects in the Mining Industry_A Comparative Study of the Mining and Extraction of Uranium, Copper and Gold*. (Master of Science Thesis: Chalmers University of Technology, Göteborg, Sweden). Retrieved April 2014, from http://publications.lib.chalmers.se/records/fulltext/85984.pdf

Noeline, B., Manohar, D., & Anirudhan, T. (2005). Kinetic and equilibrium modelling of lead (II) sorption from water and wastewater by polymerized banana stem in a batch reactor. *Separation and Purification Technology*, 45(2), 131-140.

Ok, Y. S., Lee, S. S., Jeon, W.-T., Oh, S.-E., Usman, A. R., & Moon, D. H. (2011a). Application of eggshell waste for the immobilization of cadmium and lead in a contaminated soil. *Environmental geochemistry and health*, 33(1), 31-39.

Ok, Y. S., Oh, S.-E., Ahmad, M., Hyun, S., Kim, K.-R., Moon, D. H., . . . Yang, J. E. (2010). Effects of natural and calcined oyster shells on Cd and Pb immobilization in contaminated soils. *Environmental Earth Sciences*, 61(6), 1301-1308.

Ok, Y. S., Usman, A. R., Lee, S. S., Abd El-Azeem, S. A., Choi, B., Hashimoto, Y., & Yang, J. E. (2011b). Effects of rapeseed residue on lead and cadmium availability and uptake by rice plants in heavy metal contaminated paddy soil. *Chemosphere*, 85(4), 677-682.

Papaioannou, D., Katsoulos, P., Panousis, N., & Karatzias, H. (2005). The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: A review. *Microporous and mesoporous materials*, 84(1), 161-170.

Park, J. H., Lamb, D., Paneerselvam, P., Choppala, G., Bolan, N., & Chung, J.-W. (2011). Role of organic amendments on enhanced bioremediation of heavy metal (loid) contaminated soils. *Journal of hazardous materials*, 185(2), 549-574.

Periodic table_Zinc. (2014). *Water Treatment Solution_ LENNTECH*. Retrieved April 2013, from http://www.lenntech.com/periodic/elements/zn.htm

Ruttens, A., Adriaensen, K., Meers, E., De Vocht, A., Geebelen, W., Carleer, R., . . . Vangronsveld, J. (2010). Long-term sustainability of metal immobilization by soil amendments: Cyclonic ashes versus lime addition. *Environmental Pollution*, 158(5), 1428-1434.

Schieber, A., & Saldaña, M. D. A. (2009). Potato peels: a source of nutritionally and pharmacologically interesting compounds_a review. *Food*, 3: 23-29.

Silveira, M. L. A., Alleoni, L. R. F., & Guilherme, L. R. G. (2003). Biosolids and heavy metals in soils. *Scientia Agricola*, 60(4), 793-806.

Stewart, M., Phillips, N. R., Olsen, G., Hickey, C. W., & Tipa, G. (2011). Organochlorines and heavy metals in wild caught food as a potential human health risk to the indigenous Māori population of South Canterbury, New Zealand. *Science of the Total Environment*, 409(11), 2029-2039.

Tack, F., & Verloo, M. G. (1995). Chemical speciation and fractionation in soil and sediment heavy metal analysis: a review. *International Journal of Environmental Analytical Chemistry*, 59(2-4), 225-238.

Temminghoff, E. J., Van der Zee, S. E., & de Haan, F. A. (1997). Copper mobility in a copper-contaminated sandy soil as affected by pH and solid and dissolved organic matter. *Environmental Science & Technology*, 31(4), 1109-1115.

Tessier, A., Campbell, P. G., & Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analytical chemistry*, 51(7), 844-851.

Tiinay, O., Orhon, D., & Kabdasli, I. (1994). Pretreatment requirements for leather tanning industry wastewaters. *Water Science & Technology*, 29(9), 121-128.

Toxicological Profile for Cadmium. (2010). *U.S. Department of Health and Human Services Public Health Service, Agency for Toxic Substances and Disease Registry*, Retrieved April 2013, from http://www.atsdr.cdc.gov/toxprofiles/tp5.pdf

Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *ISRN Ecology*, 2011. doi:10.5402/2011/402647.

Zhang, M.-K., Liu, Z.-Y., & Wang, H. (2010). Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. *Communications in Soil Science and Plant Analysis*, 41(7), 820-831.

Zhao, X.-l., & Masaihiko, S. (2007). Fractionation and solubility of cadmium in paddy soils amended with porous hydrated calcium silicate. *Journal of Environmental Sciences*, 19(3), 343-347.

Zhou, Y., Haynes, R., & Naidu, R. (2012). Use of inorganic and organic wastes for in situ immobilisation of Pb and Zn in a contaminated alkaline soil. *Environmental Science and Pollution Research*, 19(4), 1260-1270.

Zimmerman, A. J., & Weindorf, D. C. (2010). Heavy metal and trace metal analysis in soil by sequential extraction: a review of procedures. *International Journal of Analytical Chemistry*, 2010. doi: 10.1155/2010/387803.

Zinc and compounds. (2014). *Australian Government, Department of Sustainability, Environment, Water, Pollution and Communities*, Retrived April 2013, from http://www.npi.gov.au/resource/zinc-and-compounds

APPENDICES

APPENDIX A: Determination of Soil Texture by Hydrometer Method

Procedure:

1. Weigh 40 g air-dry soil into a 250-mL plastic bottle, add 50 mL sodium hexametaphosphate (50 g/L) solution (SHMP), add 100 mL ultrapure water, cap the bottle and place it on a mechanical shaker overt night.

Note: Sodium hexametaphosphate (50 g/L) solution prepared by dissolving 25 g of sodium hexametaphosphate in 500 mL volumetric flask.

2. Quantitatively transfer SHMP-treated soil to a 1000-mL graduated cylinder (Cylinder 1 for the soil sample). Fill to 950-mL with ultrapure water. Then add 1 to 2 drops amyl alcohol if foamy, fill to 1000-mL with ultrapure water.
3. Add 50 mL SHMP solution to a 1000-mL graduated cylinder (Cylinder 2 for the blank). Fill to 1000-mL mark with ultrapure water.
4. Thoroughly mix the soil cylinder with plunger for 1 minute, remove the plunger and mark as Time 0. Immediately insert hydrometer and read it after 40 seconds.
5. Remove the hydrometer, wipe it and take a reading of blank.
6. After 2 hours, take another soil and blank reading.

Calculation:

Original concentration = Oven-dried soil / 1000 mL

% Sand = [(Original concentration – Corrected 40 seconds reading) / Original concentration] * 100

% Clay = (Corrected 2 hours reading / Original concentration) * 100

% Silt = 100 – (% Sand + % Clay)

* Correct hydrometer reading from soil and blank cylinder:

reading time	hydrometer reading (g/L)		corrected reading (soil-blank) (g/L)
	soil cylinder	blank cylinder	
40 seconds	1	A	A - 1
2 hours	1	B	B - 1

APPENDIX B: Determination of Soil pH and Electrical Conductivity

pH Procedure:

1. Weigh 20 g air-dry soil (fraction < 2 mm) into a 100-mLplastic bottle.
2. Add 100 mL ultrapure water, cap the bottle, mix well and then place it in a mechanical shaker for 10 minutes.
3. Wait for few minutes; filter the solution through a filter paper.
4. Transfer the clear filtrate into a 100-mL beaker.
5. Put the Combined Electrode in the suspension, and take the reading.

Electrical Conductivity Procedure:

1. Prepare a 1:5 soil (w): water (v) suspension, as for pH determination.
2. After filtering the solution through a filter paper.
3. Transfer the clear filtrate into a 100-mL beaker.
4. Immerse the Conductivity Cell in the solution, and take the reading.

APPENDIX C: Determination of Soil Cation Exchange Capacity by the $BaCl_2$ Compulsive Exchange Method

Procedure:

1. Weigh 0.2 g of soil sample and transferred it into a 30 mL centrifuge tube.
2. Add 20 mL of 0.1 *M* $BaCl_2$ to the soil sample, cap, and shake it for 2 hours.

Note: 0.1 *M* $BaCl_2$ solution prepared by dissolving 2.44 g of $BaCl_2$ in 100 mL ultrapure water.

3. Centrifuge at about 10,000 rpm and decant carefully.
4. Determined Ca, Mg, K, and Al in this extract by the use of ICP.

Calculation:

CEC (cmol / kg) = [(Ca/20) + (Mg/12) + (K/39) + (Al/9)]

APPENDIX D: Determination of Soil Organic Matter by Walkley-Black Method

Procedure:

1. Weigh 1 g of air-dry soil into a 500-mL beaker.
2. Add 10 mL 1 *N* potassium dichromate solution ($K_2Cr_2O_7$), add 20 mL concentrated sulfuric acid (H_2SO_4), and swirl the beaker to mix the suspension.

Note: Preparing 1 *N* potassium dichromate was achieved by dissolving 49.04 g oven-dried potassium dichromate in 1 liter ultrapure water.

3. Allow to stand for 30 minutes.
4. Add about 200 mL ultrapure water, then add 10 mL concentrated orthophosphoric acid (H_3PO_4), add 0.2 g sodium fluoride (NaF), and allow the mixture to cool.
5. Add 10-15 drops diphenylamine indicator $(C_6H_5)_2NH$, add a Teflon-coated magnetic stirring bar, and place the beaker on a magnetic stirrer.

Note: Preparing diphenylamine indicator was achieved by dissolving 0.5 g diphenylamine in 20 mL ultrapure water. Add 100 mL sulfuric acid slowly.

6. Titrate with 0.5 *N* ferrous ammonium sulfate solution [$(NH_4)_2SO_4.FeSO_4.6H_2O$], until the color change from dull green to a turbid blue.

Note: Preparing 0.5 *N* ferrous ammonium sulfate was achieved by adding 196.1 g ferrous ammonium sulfate to a 1 volumetric flask containing 20 mL sulfuric acid and 800 mL ultrapure water. Dissolve and dilute to volume with ultrapure water.

7. Prepare two blanks, containing all reagents but no soil, and treat them in exactly the same way as the soil suspension.

Calculations:

% Oxidizable organic carbon = $[(V_B - V_S) * 0.3 * M] / Wt$

% Total organic carbon = 1.33 * % oxidizable organic carbon

% Organic matter = 1.72 * % total organic carbon

V_B = Volume of ferrous ammonium sulfate solution required to titrate the blank (mL)

V_S = Average volume of ferrous ammonium sulfate solution required to titrate the sample (mL)

$0.3 = 3 * 10^{-3} * 100$, where 3 is the equivalent weight of C.

M = Molarity of ferrous ammonium sulfate solution (approximately 0.5 M)

Wt = Weight of air-dry soil (g)

1.33 = A factor derived from the conversion of % oxidizable organic carbon to % total organic carbon.

1.72 = A factor derived from the conversion of % total organic carbon to % organic matter.

APPENDIX E: Spiking the Soil with Metal Salts

Procedure:

Four metal salts, $CdCl_2.H_2O$, $CuCl_2.2H_2O$, $Pb(C_2H_3O_2)_2.3H_2O$, and $Zn(C_2H_3O_2)_2 .2H_2O$ were used in this research. For each element, the appropriate amount of salt was brought in solution form and added to the soil.

The soil contamination was done by the following formula:

Concentration of metal salt added to per kg of the soil (mg) = Concentration of element in the soil (mg) * (Molecular weight of metal salt / Atomic weight of element)

APPENDIX F: Determination of Water Holding Capacity (WHC)

1. Attach and clamp tubing to bottom of funnel and attach the funnel to retort stand.
2. Place a filter paper in the funnel and fill the funnel with the 100 mL air-dried soil sample – do not compost.
3. Gradually add 100 mL ultrapure water to the sample until covered. Record the amount of water added.
4. Stir gently and let the soil sample to fully saturate.
5. Release the clamp and collect the water in a graduated cylinder.
6. Record the amount of water in the cylinder.

Calculation:

Volume of water retained (mL) = volume of water added to the soil sample (mL) – volume of water collected (mL)

% Water holding capacity = volume of water retained (mL) / 100 mL soil sample

APPENDIX G: Digestion Method of Soil

Procedure:

About 0.5 g of well-mixed soil sample was transferred into the microwave vessels. About 9 mL concentrated nitric acid (HNO_3), 3 mL concentrated hydrofluoric acid (HF), and 2 mL concentrated hydrochloric acid (HCl) were added to the sample. For each digestion, sample prepared in 3 replicates with one blank contained only reagents. The temperature of each sample was raised to 175 ± 5 ^{o}C approximately in 4.5 minutes and remained at 175 ± 5 ^{o}C for 5.5 minutes, with the cooling time of 15 minutes. The power for the digestion was set to 1000 W. After digestion completed and samples cooled at room temperature, the sample digests were filtered with a Whatman No. 1 filter paper, transferred into a 100-mL volumetric flask and brought to volume with ultrapure water. The digested sample along with the blank was analyzed using ICP-OES spectrometer. The standard solutions of metals prepared daily.

Note: The accuracy and precision of microwave digestion methods are calculated using the formula below:

Percentage Recovery = (concentration of element / original concentration of CRM) * 100

[The measured concentration values might be in agreement with required quality control criteria which is 80-120 percent.]

APPENDIX H: Digestion Method of Amendments

Procedure:

About 0.5 g of oven-dried amendment was added with 9 ml nitric acid (HNO_3) and digested in the microwave oven. Samples prepared in 3 replicate with one blank contained only reagents. The temperature was set at 170 ± 5 ^{o}C for 15 minutes with the power of 800 W. After cooling time, digested sample was filtered using Whatman No. 1, transferred to the 100-mL volumetric flask and brought to volume. Both sample and blank were analyzed using ICP-OES spectrometer.

APPENDIX I: pH & metal concentrations in the leachates of treatments

Week 2

Amendment	Replicate	pH	Pb *	Cd *	Zn *	Cu *	Al *	Fe *
Banana Stem	1.00	7.60	.83	.09	1.64	1.57	.05	.18
Banana Stem	2.00	7.40	.58	.09	1.75	1.10	.01	.28
Banana Stem	3.00	7.50	1.00	.12	2.23	2.31	.18	.35
Potato Peel	1.00	7.60	12.40	.51	19.76	36.85	4.54	14.35
Potato Peel	2.00	7.70	16.14	.69	20.73	48.65	4.69	18.61
Potato Peel	3.00	7.90	26.66	.90	29.57	55.07	7.22	35.29
Coconut Husk	1.00	6.56	.19	1.52	30.01	.29	.02	.03
Coconut Husk	2.00	6.50	.17	1.72	34.82	.24	.30	.11
Coconut Husk	3.00	6.50	.17	2.12	40.49	.26	.11	.04
Eggshell	1.00	7.30	.07	.24	2.35	.31	.00	.00
Eggshell	2.00	7.30	.09	.29	2.58	.33	.00	.00
Eggshell	3.00	7.60	.08	.28	1.97	.39	.00	.00
Control	1.00	7.40	.09	1.23	5.99	.18	.00	.00
Control	2.00	7.20	.08	1.36	8.54	.17	.00	.00
Control	3.00	7.20	.10	1.89	8.81	.28	.00	.00

*: mg kg^{-1}

Week 4

Amendment	Replicate	pH	Pb *	Cd *	Zn *	Cu *	Al *	Fe *
Banana Stem	1.00	7.60	3.07	.32	5.44	4.55	1.31	2.37
Banana Stem	2.00	7.70	1.12	.16	2.73	2.27	.24	.69
Banana Stem	3.00	7.50	3.12	.32	5.17	4.63	.38	1.14
Potato Peel	1.00	7.95	33.86	1.73	63.85	89.43	19.53	46.57
Potato Peel	2.00	7.94	20.12	1.03	28.59	55.63	8.63	25.94
Potato Peel	3.00	8.04	40.53	1.87	47.52	96.54	18.52	64.09
Coconut Husk	1.00	6.66	.19	1.41	29.38	.24	.25	.15
Coconut Husk	2.00	6.61	.20	1.60	34.36	.32	.76	.29
Coconut Husk	3.00	6.63	.22	1.60	33.28	.19	.32	.14
Eggshell	1.00	7.50	.08	.11	1.13	.17	.00	.02
Eggshell	2.00	7.60	.07	.08	.87	.16	.00	.01
Eggshell	3.00	7.70	.09	.08	.77	.19	.00	.00
Control	1.00	7.50	.08	.59	3.34	.11	.00	.04
Control	2.00	7.50	.05	.68	4.54	.11	.02	.03
Control	3.00	7.50	.04	.59	2.10	.18	.00	.00

*: mg kg^{-1}

Week 6

Amendment	Replicate	pH	Pb *	Cd *	Zn *	Cu *	Al *	Fe *
Banana Stem	1.00	7.40	.53	.07	1.11	.98	.65	.55
Banana Stem	2.00	7.70	.28	.08	1.04	.61	.06	.11
Banana Stem	3.00	7.50	.60	.08	1.32	1.40	.07	.15
Potato Peel	1.00	7.86	11.97	.55	17.23	38.79	7.78	13.48
Potato Peel	2.00	7.64	6.79	.32	8.21	21.50	2.84	6.96
Potato Peel	3.00	7.65	20.32	.83	20.67	50.55	9.47	26.25
Coconut Husk	1.00	6.50	.23	.90	22.13	.32	.48	.19
Coconut Husk	2.00	6.48	.52	.94	23.21	.38	3.34	1.07
Coconut Husk	3.00	6.44	.35	.90	23.05	.37	6.41	2.05
Eggshell	1.00	7.30	.05	.05	1.17	.16	.08	.04
Eggshell	2.00	7.40	.02	.03	.47	.16	.03	.02
Eggshell	3.00	7.70	.03	.03	.40	.18	.03	.02
Control	1.00	7.40	.02	.23	1.80	.11	.06	.03
Control	2.00	7.30	.04	.26	3.03	.10	.34	.13
Control	3.00	7.30	.11	.27	1.66	.14	.05	.02

*: mg kg^{-1}

Week 8

Amendment	Replicate	pH	Pb *	Cd *	Zn *	Cu *	Al *	Fe *
Banana Stem	1.00	7.40	.73	.07	1.36	.82	.09	.32
Banana Stem	2.00	7.40	.14	.02	.55	.30	.02	.09
Banana Stem	3.00	7.50	.61	.04	1.03	1.06	.04	.22
Potato Peel	1.00	7.60	10.86	.55	13.58	29.19	1.72	10.28
Potato Peel	2.00	7.70	2.12	.11	2.86	5.85	.14	2.29
Potato Peel	3.00	7.54	14.59	.62	14.93	33.56	3.41	20.76
Coconut Husk	1.00	6.60	.59	.38	12.06	.32	.28	.36
Coconut Husk	2.00	6.57	.19	.43	13.26	.22	.87	.81
Coconut Husk	3.00	6.50	.25	.45	13.81	.26	1.65	1.64
Eggshell	1.00	7.50	.06	.03	.83	.09	.07	.10
Eggshell	2.00	7.40	.05	.01	.18	.11	.07	.09
Eggshell	3.00	7.70	.04	.01	.29	.11	.04	.07
Control	1.00	7.70	.03	.05	.75	.08	.08	.13
Control	2.00	7.60	.06	.10	1.63	.07	.26	.32
Control	3.00	7.50	.04	.11	1.19	.08	.05	.06

*: mg kg^{-1}

Week 10

Amendment	Replicate	pH	Pb *	Cd *	Zn *	Cu *	Al *	Fe *
Banana Stem	1.00	7.40	.45	.05	1.00	.77	.02	.08
Banana Stem	2.00	7.40	.15	.02	.48	.30	.01	.04
Banana Stem	3.00	7.30	.52	.03	.86	.99	.03	.13
Potato Peel	1.00	7.64	9.01	.54	12.93	28.29	4.00	8.49
Potato Peel	2.00	7.55	1.26	.06	1.53	4.68	.07	1.31
Potato Peel	3.00	7.50	9.15	.43	9.93	23.05	3.70	10.27
Coconut Husk	1.00	6.94	.34	.13	4.89	.32	1.25	.69
Coconut Husk	2.00	6.64	.14	.19	6.98	.26	.36	.18
Coconut Husk	3.00	6.72	.36	.23	7.80	.37	3.46	1.64
Eggshell	1.00	7.60	.02	.01	.24	.10	.01	.00
Eggshell	2.00	7.80	.03	.00	.08	.11	.01	.01
Eggshell	3.00	7.80	.02	.00	.04	.11	.00	.00
Control	1.00	7.80	.02	.02	.23	.08	.13	.04
Control	2.00	7.70	.04	.06	.76	.06	.01	.01
Control	3.00	7.70	.06	.08	.80	.10	.04	.04

*: mg kg^{-1}

Week 12

Amendment	Replicate	pH	Pb *	Cd *	Zn *	Cu *	Al *	Fe *
Banana Stem	1.00	7.50	.54	.05	1.13	.91	.12	.24
Banana Stem	2.00	7.60	.23	.03	.63	.42	.03	.06
Banana Stem	3.00	7.20	.63	.05	1.32	1.01	.17	.33
Potato Peel	1.00	7.65	10.10	.56	15.24	23.06	8.66	13.57
Potato Peel	2.00	7.32	1.33	.07	1.65	4.32	.19	1.45
Potato Peel	3.00	7.53	10.26	.52	14.10	21.39	7.74	13.72
Coconut Husk	1.00	6.84	.36	.07	3.45	.46	4.31	1.83
Coconut Husk	2.00	6.68	.33	.09	4.19	.40	2.80	1.16
Coconut Husk	3.00	6.74	.32	.09	4.18	.46	8.34	2.77
Eggshell	1.00	7.60	.04	.01	.39	.09	.01	.00
Eggshell	2.00	7.60	.03	.01	.17	.11	.01	.01
Eggshell	3.00	7.90	.05	.01	.22	.10	.00	.00
Control	1.00	7.60	.04	.02	.30	.07	.11	.06
Control	2.00	7.50	.05	.07	1.03	.06	.05	.02
Control	3.00	7.50	.07	.08	.89	.11	.15	.11

*: mg kg^{-1}

APPENDIX J: Determination of significant differences of pH in leachates of different treatments

Tests of Normality							
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Amendment		Statistic	df	Sig.	Statistic	df	Sig.
pH	Banana Stem	.168	18	.192	.940	18	.290
	Potato Peel	.188	18	.093	.943	18	.322
	Coconut Husk	.146	18	.200*	.928	18	.176
	Eggshell	.172	18	.168	.943	18	.323
	Control	.179	18	.130	.947	18	.381

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Test of Homogeneity of Variances			
Levene Statistic	df1	df2	Sig.
.886	4	85	.476

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.197	4	3.299	124.984	.000
Within Groups	2.244	85	.026		
Total	15.441	89			

Multiple Comparisons						
Dependent Variable: pH						
POST-HOC (Tukey HSD)						
					95% Confidence Interval	
(I) Amendment	(J) Amendment	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Banana Stem	Control	-.01667	.05416	.998	-.1676	.1343
Potato Peel	Control	.18944*	.05416	.007	.0385	.3404
Coconut Husk	Control	-.87722*	.05416	.000	-1.0282	-.7263
Eggshell	Control	.07778	.05416	.606	-.0732	.2287

*. The mean difference is significant at the 0.05 level.

Pairwise Comparisons							
Dependent Variable: pH			Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
Week	(I) Amendment	(J) Amendment				Lower Bound	Upper Bound
Week 2	Banana Stem	Control	.233*	.094	.015	.046	.421
	Potato Peel	Control	.467*	.094	.000	.279	.654
	Coconut Husk	Control	-.747*	.094	.000	-.934	-.559
	Eggshell	Control	.133	.094	.159	-.054	.321
Week 4	Banana Stem	Control	.100	.094	.289	-.087	.287
	Potato Peel	Control	.477*	.094	.000	.289	.664
	Coconut Husk	Control	-.867*	.094	.000	-1.054	-.679
	Eggshell	Control	.100	.094	.289	-.087	.287
Week 6	Banana Stem	Control	.200*	.094	.037	.013	.387
	Potato Peel	Control	.383*	.094	.000	.196	.571
	Coconut Husk	Control	-.860*	.094	.000	-1.047	-.673
	Eggshell	Control	.133	.094	.159	-.054	.321
Week 8	Banana Stem	Control	-.167	.094	.080	-.354	.021
	Potato Peel	Control	.013	.094	.887	-.174	.201
	Coconut Husk	Control	-1.043*	.094	.000	-1.231	-.856
	Eggshell	Control	-.067	.094	.479	-.254	.121
Week 10	Banana Stem	Control	-.367*	.094	.000	-.554	-.179
	Potato Peel	Control	-.170	.094	.074	-.357	.017
	Coconut Husk	Control	-.967*	.094	.000	-1.154	-.779
	Eggshell	Control	-1E-16	.094	1.000	-.187	.187
Week 12	Banana Stem	Control	-.100	.094	.289	-.287	.087
	Potato Peel	Control	-.033	.094	.723	-.221	.154
	Coconut Husk	Control	-.780*	.094	.000	-.967	-.593
	Eggshell	Control	.167	.094	.080	-.021	.354

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

APPENDIX K: Determination of significant differences of heavy metals in leachates of different treatments

Pairwise Comparisons								
Dependent Variable	Week	(I) Amendment	(J) Amendment	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
							Lower Bound	Upper Bound
Log_Pb	Week 2	Banana Stem	Control	.942*	.192	.000	.557	1.326
		Potato Peel	Control	2.292*	.192	.000	1.908	2.677
		Coconut Husk	Control	.299	.192	.124	-.085	.684
		Eggshell	Control	-.048	.192	.804	-.432	.336
	Week 4	Banana Stem	Control	1.601*	.192	.000	1.216	1.985
		Potato Peel	Control	2.738*	.192	.000	2.354	3.123
		Coconut Husk	Control	.570*	.192	.004	.185	.954
		Eggshell	Control	.161	.192	.407	-.224	.545
	Week 6	Banana Stem	Control	.987*	.192	.000	.603	1.372
		Potato Peel	Control	2.411*	.192	.000	2.026	2.795
		Coconut Husk	Control	.878*	.192	.000	.494	1.263
		Eggshell	Control	-.140	.192	.468	-.525	.244
	Week 8	Banana Stem	Control	.952*	.192	.000	.568	1.337
		Potato Peel	Control	2.193*	.192	.000	1.809	2.578
		Coconut Husk	Control	.839*	.192	.000	.455	1.224
		Eggshell	Control	.040	.192	.835	-.344	.425
	Week 10	Banana Stem	Control	.950*	.192	.000	.565	1.334
		Potato Peel	Control	2.105*	.192	.000	1.720	2.489
		Coconut Husk	Control	.847*	.192	.000	.463	1.232
		Eggshell	Control	-.166	.192	.391	-.550	.218
	Week 12	Banana Stem	Control	.905*	.192	.000	.520	1.289
		Potato Peel	Control	1.991*	.192	.000	1.606	2.375
		Coconut Husk	Control	.807*	.192	.000	.422	1.191
		Eggshell	Control	-.138	.192	.475	-.523	.246
Log_Cd	Week 2	Banana Stem	Control	-1.171*	.186	.000	-1.543	-.798
		Potato Peel	Control	-.333	.186	.079	-.705	.039
		Coconut Husk	Control	.081	.186	.667	-.292	.453
		Eggshell	Control	-.735*	.186	.000	-1.107	-.362
	Week 4	Banana Stem	Control	-.384*	.186	.043	-.757	-.012
		Potato Peel	Control	.381*	.186	.045	.009	.754
		Coconut Husk	Control	.393*	.186	.039	.021	.766
		Eggshell	Control	-.837*	.186	.000	-1.209	-.464
	Week 6	Banana Stem	Control	-.512*	.186	.008	-.884	-.139

		Potato Peel	Control	.323	.186	.088	-.050	.695
		Coconut Husk	Control	.559*	.186	.004	.187	.932
		Eggshell	Control	-.822*	.186	.000	-1.194	-.449
	Week 8	Banana Stem	Control	-.345	.186	.069	-.718	.027
		Potato Peel	Control	.617*	.186	.002	.244	.989
		Coconut Husk	Control	.711*	.186	.000	.339	1.083
		Eggshell	Control	-.835*	.186	.000	-1.208	-.463
	Week 10	Banana Stem	Control	-.097	.186	.604	-.470	.275
		Potato Peel	Control	.739*	.186	.000	.367	1.111
		Coconut Husk	Control	.606*	.186	.002	.234	.979
		Eggshell	Control	-.930*	.186	.000	-1.302	-.558
	Week 12	Banana Stem	Control	-.084	.186	.653	-.457	.288
		Potato Peel	Control	.735*	.186	.000	.362	1.107
		Coconut Husk	Control	.235	.186	.212	-.137	.608
		Eggshell	Control	-.656*	.186	.001	-1.029	-.284
Log_Zn	Week 2	Banana Stem	Control	-.616*	.189	.002	-.995	-.237
		Potato Peel	Control	.477*	.189	.015	.098	.855
		Coconut Husk	Control	.658*	.189	.001	.279	1.036
		Eggshell	Control	-.526*	.189	.007	-.904	-.147
	Week 4	Banana Stem	Control	.127	.189	.504	-.251	.506
		Potato Peel	Control	1.145*	.189	.000	.766	1.523
		Coconut Husk	Control	1.007*	.189	.000	.629	1.386
		Eggshell	Control	-.543*	.189	.006	-.922	-.164
	Week 6	Banana Stem	Control	-.256	.189	.181	-.635	.123
		Potato Peel	Control	.837*	.189	.000	.458	1.216
		Coconut Husk	Control	1.040*	.189	.000	.661	1.418
		Eggshell	Control	-.537*	.189	.006	-.916	-.158
	Week 8	Banana Stem	Control	-.093	.189	.624	-.472	.285
		Potato Peel	Control	.866*	.189	.000	.487	1.245
		Coconut Husk	Control	1.060*	.189	.000	.681	1.438
		Eggshell	Control	-.507*	.189	.010	-.885	-.128
	Week 10	Banana Stem	Control	.158	.189	.408	-.221	.536
		Potato Peel	Control	1.051*	.189	.000	.672	1.430
		Coconut Husk	Control	1.095*	.189	.000	.716	1.474
		Eggshell	Control	-.783*	.189	.000	-1.162	-.404
	Week 12	Banana Stem	Control	.176	.189	.355	-.202	.555
		Potato Peel	Control	1.035*	.189	.000	.656	1.414
		Coconut Husk	Control	.779*	.189	.000	.400	1.158
		Eggshell	Control	-.426*	.189	.028	-.805	-.047
Log_Cu	Week 2	Banana Stem	Control	.892*	.148	.000	.597	1.187
		Potato Peel	Control	2.357*	.148	.000	2.062	2.652
		Coconut Husk	Control	.110	.148	.458	-.185	.405
		Eggshell	Control	.223	.148	.135	-.072	.518
	Week 4	Banana Stem	Control	1.436*	.148	.000	1.141	1.732
		Potato Peel	Control	2.771*	.148	.000	2.476	3.066

		Coconut Husk	Control	.261	.148	.082	-.034	.556
		Eggshell	Control	.113	.148	.446	-.182	.408
	Week 6	Banana Stem	Control	.911*	.148	.000	.616	1.207
		Potato Peel	Control	2.479*	.148	.000	2.184	2.774
		Coconut Husk	Control	.488*	.148	.002	.193	.783
		Eggshell	Control	.162	.148	.277	-.133	.457
	Week 8	Banana Stem	Control	.910*	.148	.000	.615	1.206
		Potato Peel	Control	2.356*	.148	.000	2.061	2.651
		Coconut Husk	Control	.519*	.148	.001	.224	.814
		Eggshell	Control	.108	.148	.468	-.187	.403
	Week 10	Banana Stem	Control	.901*	.148	.000	.606	1.196
		Potato Peel	Control	2.275*	.148	.000	1.980	2.570
		Coconut Husk	Control	.613*	.148	.000	.318	.909
		Eggshell	Control	.136	.148	.362	-.159	.431
	Week 12	Banana Stem	Control	.985*	.148	.000	.690	1.280
		Potato Peel	Control	2.231*	.148	.000	1.936	2.526
		Coconut Husk	Control	.763*	.148	.000	.468	1.058
		Eggshell	Control	.113	.148	.448	-.183	.408

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Appendix L: Determination of significant differences of heavy metals in soil in different treatments

Pairwise Comparisons								
Dependent Variable	Sequential Extraction	(I) Amendment	(J) Amendment	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
							Lower Bound	Upper Bound
log_Pb	Exchangeable	Banana Stem	Control	-.545*	.130	.000	-.810	-.280
		Eggshell	Control	-.487*	.130	.001	-.752	-.222
	Carbonate	Banana Stem	Control	-.253	.130	.061	-.518	.012
		Eggshell	Control	-.002	.130	.990	-.267	.263
	Fe_Mn	Banana Stem	Control	.139	.130	.294	-.127	.404
		Eggshell	Control	-.069	.130	.598	-.334	.196
	Oraginc Matter	Banana Stem	Control	.575*	.130	.000	.310	.840
		Eggshell	Control	-.356*	.130	.010	-.621	-.091
	Residual	Banana Stem	Control	-.319*	.130	.020	-.584	-.054
		Eggshell	Control	.175	.130	.188	-.090	.440
log_Cd	Exchangeable	Banana Stem	Control	-.234	.157	.146	-.554	.086
		Eggshell	Control	-.142	.157	.372	-.462	.178
	Carbonate	Banana Stem	Control	.068	.157	.667	-.252	.388
		Eggshell	Control	.467*	.157	.006	.147	.787
	Fe_Mn	Banana Stem	Control	.039	.157	.803	-.281	.359
		Eggshell	Control	-.091	.157	.566	-.411	.229
	Oraginc Matter	Banana Stem	Control	-.338*	.157	.039	-.658	-.018
		Eggshell	Control	-.164	.157	.304	-.484	.156
		Control	Eggshell	.164	.157	.304	-.156	.484
	Residual	Banana Stem	Control	.036	.157	.820	-.284	.356
		Eggshell	Control	-.037	.157	.816	-.357	.283
log_Zn	Exchangeable	Banana Stem	Control	-.143	.116	.227	-.380	.094
		Eggshell	Control	-.339*	.116	.007	-.576	-.102
	Carbonate	Banana Stem	Control	-.153	.116	.198	-.390	.084
		Eggshell	Control	-.011	.116	.926	-.248	.226
	Fe_Mn	Banana Stem	Control	.127	.116	.283	-.110	.364
		Eggshell	Control	-.030	.116	.795	-.268	.207
	Oraginc Matter	Banana Stem	Control	.025	.116	.831	-.212	.262
		Eggshell	Control	-.390*	.116	.002	-.628	-.153
	Residual	Banana Stem	Control	-.124	.116	.293	-.361	.113
		Eggshell	Control	.066	.116	.572	-.171	.303
log_Cu	Exchangeable	Banana Stem	Control	.051	.084	.546	-.121	.224
		Eggshell	Control	-.245*	.084	.007	-.417	-.072
	Carbonate	Banana Stem	Control	-.667*	.084	.000	-.839	-.495

		Eggshell	Control	-.071	.084	.406	-.243	.101
	Fe_Mn	Banana Stem	Control	.090	.084	.292	-.082	.263
		Eggshell	Control	.008	.084	.922	-.164	.180
	Oraginc Matter	Banana Stem	Control	.561*	.084	.000	.388	.733
		Eggshell	Control	-.123	.084	.154	-.295	.049
	Residual	Banana Stem	Control	.059	.084	.489	-.113	.231
		Eggshell	Control	-.099	.084	.250	-.271	.073

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

LIST OF PUBLICATION AND PRESENTATION

Publication:

1. Mehrnaz Ashrafi*, Sharifah Mohamad, Ismail Yusoff, Fauziah Shahul Hamid, "Immobilization of Pb, Cd and Zn in a contaminated soil using eggshell and banana stem amendments: Metal leachability and a sequential extraction study", *Environmental Science and Pollution Research*. DOI: 10.1007/s11356-014-3299-4.

Seminar:

1. Poster presentation in "5th UM-NUS-CU Trilateral mini Symposium and Scientific Meeting 2014", 11 - 12 February 2014, University of Malaya, Malaysia.

2. Best poster award in "UK-Malaysia Joint Symposium on Inorganic Chemistry", 5 December 2013, University of Malaya, Malaysia.

3. Poster presentation in "International Conference on Waste Management and Environment 2013", 26 - 27 August 2013, University of Malaya, Malaysia.

4. Attended "SNIC-RSC Joint Symposium on Inorganic Chemistry", 7 - 8 January 2013, Institute of Materials Research and Engineering, Singapore.